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ON THE IMPOSSIBILITY OF ALL OVERLAPPING TRIPLET CODES IN INFORMATION TRANSFER FROM NUCLEIC ACID TO PROTEINS

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Communicated by G. Gamow, June 10, 1957

It is a generally accepted view that nucleic acids control the synthesis of proteins, and it has been proposed more specifically that the sequence of amino acids in a polypeptide chain is determined by the order of nucleotides in ribo- or deoxyribonucleic acid. The problem of how this determination is effected has come to be known as the "coding" problem. The formal aspects of this problem can be investigated theoretically, and most of the work done in this field has recently been reviewed by Gamow, Rich, and Yčas.[1]

Since there are only four different nucleotides in RNA or DNA to determine twenty different amino acids, it is clear that more than one nucleotide must be used to code for each amino acid. Most codes have been constructed on the basis that each amino acid is determined by a set of three nucleotides. Such triplet codes, however, have an excess of information, since there are sixty-four different triplets for the twenty amino acids. In Gamow's original diamond code,[2] several triplets, chosen in a particular way, coded for any given amino acid; the code was therefore "degenerate." This code was also of the overlapping type—that is, the number of nucleotides in the nucleic acid was equal to the number of amino acids in the polypeptide chain. Gamow's diamond code does not, in fact, code for known sequences, and the same is true for the major-minor code, another overlapping triplet code, invented by L. Orgel.[1] These are, however, only two examples of a large number of possible codes of this type which can be obtained by choosing different ways of degenerating the triplets. To test all of these systematically is clearly impossible, and hence it is necessary to have some general theorem about such codes.

The general overlapping triplet code has the following properties.

(i) The coding triplets are chosen from four nucleotides, *A*, *B*, *C*, and *D*, giving sixty-four different triplets.

(ii) Coding is overlapping, each triplet sharing two nucleotides with the succeeding triplet in a sequence. Thus the sequence *ABCDA* codes for three amino acids: *ABC* for the first, *BCD* for the second, and *CDA* for the third.

(iii) An amino acid may be represented by more than one triplet; that is, the sixty-four triplets are degenerated into twenty sets.

Since any dipeptide sequence is represented by a sequence of four nucleotides, there cannot be more than 256 different dipeptides. On the other hand, if all dipeptide sequences were possible, 400 would be expected. Thus overlapping codes introduce restrictions in amino acid sequences. The number of dipeptide sequences known is less than 256, and, although statistical studies have suggested that all dipeptides are likely to be found, the significance of this result has been difficult to assess.[1] The sample of proteins studied is highly selected, a large number of sequences are fragmentary, and the methods used to study sequences further bias the data.

However, sufficient sequences are known to prove that it is impossible to code them with overlapping triplets. The proof is simple and does not depend on any special way of degenerating the triplets. It consists in the demonstration that sixty-four triplets are insufficient to code the known sequences.

Proof: Since successive triplets share two nucleotides in common, any given triplet can be preceded by only four different triplets and succeeded by only four different triplets. In an amino acid sequence *j.k.l.*, we call *j* an N-neighbor, and *l* a C-neighbor, of *k*. For every four different N-neighbors (or C-neighbors) or part thereof, *k* must have one triplet assigned to it. Thus the *minimum* number of triplet representations for each amino acid can be counted from a table of neighbors.

The available sequences are given in the Appendix. From these sequences a grid is constructed and the different neighbors counted for each amino acid. The number of triplets assigned to each amino acid is based on the larger number of its neighbors. These data are given in Table 1, from which it can be seen that seventy

TABLE 1

Amino Acid	C-Neighbors	N-Neighbors	Minimum No. of Triplets Required	Amino Acid	C-Neighbors	N-Neighbors	Minimum No. of Triplets Required
Lys	18	17	5	Pro	13	12	4
Ser	17	13	5	Tyr	12	10	3
Gly	15	15	4	Glu	11	11	3
Leu	15	15	4	Glun	12	9	3
Cys	15	14	4	Asp	10	11	3
Arg	14	16	4	Asn	9	10	3
Ala	14	15	4	Ileu	9	9	3
Val	14	12	4	His	6	9	3
Thr	13	14	4	Met	5	7	2
Phe	13	14	4	Try	3	3	1
						Total	70

triplets would be required to code the sequences. We conclude, then, that all overlapping triplet codes are impossible.

This result has one important physical implication. The original formulation of overlapping codes was based on the similarity of the internucleotide distance in DNA to the spacing between amino acid residues in an extended polypeptide chain. It was supposed that each amino acid was spatially related in a one-to-one way with each nucleotide on a nucleic acid template. The present result shows that this cannot be so and that each amino acid is stereochemically related to at least two, if not three, nucleotides, depending on whether coding is partially overlapping or nonoverlapping. The difficulties raised by this can easily be overcome by assuming that the polypeptide sequence is in contact with the nucleic acid template only at the growing point, and detailed schemes can be readily proposed.

As far as the coding problem is concerned, it now appears that all amino sequences are likely to be found and that it will not be possible to effect a "decoding" by discovering restrictions in sequences. The nonoverlapping of triplets implies that there must be some way of determining which triplets in a sequence are coding triplets and which are not, and a very interesting code has recently been proposed by Crick, Griffith, and Orgel,[3] in which this problem is dealt with in a novel manner.

APPENDIX

AMINO ACID SEQUENCES

In writing the sequences, the same conventions used by Gamow *et al.*[1] have been followed. Wherever doubt exists as to whether glutamic acid is present as such (glu) or as the amide (glun), it has been assigned as "glux," and the same rule has been followed for aspartic acid and asparagine. All the longer lysozyme sequences suggested by Thompson (*Biochem. J.*, **60,** 507; **61,** 253, 1955) have been omitted, since some of these appear to be incorrect when compared with those established by the French workers. Sequences established by carboxypeptidase digestions alone are given at the end of the list but are omitted from the grid. The same applies to the pepsin sequence of Williamson and Passmann (*J. Biol. Chem.*, **222,** 151, 1956), as there are conflicting reports about the N-terminal group (Van Vunakis and Herriott, *Biochim. et Biophys. Acta*, **23,** 60, 1957).

The grid (Table 2) shows the number of times dipeptide sequences are found. Identical sequences from the closely related proteins vasopressin and oxytocin and corticotrophin and melanophore-stimulating hormone are only recorded once. Dipeptide sequences from lysozyme are not recorded if the same sequence is found in a longer peptide. When both glu and glun are absent, glux is counted as a neighbor, and the same rule is followed for asp, asn, and ax.

SEQUENCES USED IN THE GRID*

-thr. gly. ileu[e]-
-thr. ser. ileu[c, d]-
-ala. gly. val[b]-

Insulin A: Gly. ileu. val. glu. glun. cys. cys. ala. ser. val[a]. cys. ser. leu. tyr. glun. leu. glu. asn. tyr. cys. asn.[4–6]

Insulin B: Phe. val. asn. glun. his. leu. cys. gly. ser. his. leu. val. glu. ala. leu. tyr. leu. val. cys. gly. glu. arg. gly. phe. phe. tyr. thr. pro. lys. ala.[4, 5]

Oxytocin: Cys. tyr. ileu. glun. asn. cys. pro. leu. gly. NH_2.[7]

-arg[a]-

Vasopressin: Cys. tyr. phe. glun. asn. cys. pro. lys[b]. gly. NH_2.[8]

Corticotrophin: Ser. tyr. ser. met. glu. his. phe. arg. try. gly. lys. pro. val. gly. lys. lys. arg-arg. pro. val. lys. val. tyr. pro. *asp. gly. ala. glu.*[9] asp. glun. leu. ala[b]. glu. ala. phe. pro. leu-
-glux. ala. ser[c]-
glu. phe.[10–13]

Glucagon: His. ser. glun. gly. thr. phe. thr. ser. asp. thr. ser. lys. tyr. leu. asp. ser. arg. arg. ala. glun. asp. phe. val. glun. try. leu. met. asn. thr.[14]

-ser[a]-

Melanophore-stimulating hormone: Asp. glu[b]. gly. pro. tyr. lys. met. glu. his. phe. arg. try. gly. ser. pro. pro. lys. asp.[15–17]

* [a] Cattle. [b] Pig. [c] Sheep. [d] Whale. [e] Horse. [f] Salmon. [g] Chicken. [h] Man.

TABLE 2

Grid Showing Numbers of N- and C- Neighbors

C-Neighbors	N-Neighbors: Lys	Ser	Gly	Leu	Cys	Arg	Ala	Val	Thr	Phe	Pro	Tyr	Glu	Glun	Glux	Asp	Asn	Asx	Ileu	His	Met	Try
Lys	1	1	3	1	2	1	4	2	2	2	4	2	1	1	1	1	..	1	..	1	2	..
Ser	1	..	4	1	2	..	3	1	5	..	..	1	2	1	1	3	1	1	..	1	..	..
Gly	3	4	..	2	4	4	2	2	2	1	1	1	1	1	..	1	..	1	..	..	..	1
Leu	2	3	3	1	..	2	3	2	1	..	2	2	..	3	..	..	1	1	1	3	..	1
Cys	1	1	2	2	1	2	2	5	1	1	..	2	..	2	..	..	1	2	1	..	..	..
Arg	3	5	1	1	1	2	1	..	..	2	1	2	1	1	..	3	2	..	1	..	..	1
Ala	1	2	1	2	4	1	4	3	2	..	1	..	4	1	1	1	3	2	1	..	..	..
Val	2	3	2	4	1	1	..	..	1	2	3	1	..	..	..	..	1	..	4	..	..	..
Thr	1	2	1	1	1	..	2	1	1	1	..	2	1	..	..	1	2	1	..	1	..	..
Phe	2	1	1	..	1	1	1	1	1	1	1	1	1	..	..	1	..	..	..	1	..	..
Pro	2	3	?	1	1	1	..	..	4	1	1	2	..	..	..	..	..	..	1	1	..	..
Tyr	2	2	1	2	2	..	1	2	..	1	1	..	..	..	..	..	1	1	..	..	..	..
Glu	1	1	1	2	1	2	2	3	..	1	..	..	..	..	..	2	..	..	..	..	1	..
Glun	..	2	..	..	..	..	2	3	..	2	..	2	1	..	..	1	3	..	1	..	..	..
Glux	..	1	..	..	1	..	1	2	1	1	1	..	..	..	..	1	..	1	1	..	..	..
Asp	2	1	1	1	..	..	..	1	1	2	1	..	1	1	..	3	..	..	..	..	..	..
Asn	2	..	..	1	3	1	..	1	2	..	1	..	1	1	..	..	..	..	..	..	1	..
Asx	1	2	1	..	2	1	..	3	..	..	..	..	..	..	1	..	..	1	1	..	1	..
Ileu	1	1	2	..	1	1	..	..	..	..	1	3	..	..	1	..	..	1	..	..	..	..
His	1	1	..	..	1	1	..	1	..	1	..	..	1	1	..	..	..	..	1	..	..	..
Met	1	1	1	1	..	..	1	..	..	..	..	..	..	1	..	..	..	..	..	..	1	..
Try	..	..	..	..	..	1	1	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..

-ileu^{e}-

Hypertensin: Asp. arg. val. tyr. val^{a}. his. pro. phe. his. leu.[18, 19]

-ser^{g}-

Cytochrome c: -val. glun. lys. cys. ala$^{a, b, e, f}$. glun. cys. his. thr. val. glu. lys-[20, 21]

Trypsinogen: Val. asp. asp. asp. asp. lys. ileu. val. gly-[22, 23]

Ribonuclease: Lys. glu. thr. ala. ala. ala. lys. phe. glun. arg. glu-[24–26]

-tyr. cys. asn. glun. met. met. lys. ser. arg. asn. leu. thr. lys. asp. arg. cys.[24–27]

-lys. asn. val. ala. cys. lys. asn. thr-[26, 27]

-cys. asn. arg. glu. ser. thr. ser. gly. lys. tyr. pro. asn. ala. cys. tyr. lys. thr. thr. asn. glun. ala. lys. his-[26, 27]

-tyr. glun. ser. tyr-[24]

-phe. asp. ala. ser. val.[24, 28, 29]

Lysozyme: -lys. asx-[30]

-arg. his. lys-[31]

Lys. val. phe. gly. arg-

-ala. lys. phe. glux-

-asx. tyr. arg. gly-

-arg. gly. tyr. ileu. leu-

-asn. ala. tyr. gly. ser. leu. asn-

-thr. pro-

-leu. pro-[32]

-asn. arg-

-ileu. arg-

-thr. pro. gly. ser. arg-

-val. ala. try. arg-[33]

-gly. cys. arg. leu.

-phe. glu. ser. phe. asp. glu. ala. thr. asp. arg-

-cys. glu. ala. leu. ala. ala. met. lys. arg-[34]

-ala. ala-	-asx. ileu-	-ileu. arg-	-ser. ala-
-ala. leu-	-asx. leu-	-ileu. asx-	-ser. arg-
-ala. lys-	-cys. ala-	-ileu. val-	-ser. asx-
-ala. met-	-cys. arg-	-leu. ala-	-ser. leu-
-arg. asx-	-cys. asx-	-leu. cys-	-ser. val-
-arg. cys-	-cys. glux-	-leu. leu-	-thr. ala-
-arg. gly-	-cys. ileu-	-lys. gly-	-thr. asx-
-arg. leu-	-cys. lys-	-met. asx-	-thr. gly-
-asx. ala-	-gly. leu-	-met. lys-	-thr. glux-
-asx. arg-	-gly. met-	-phe. asx-	-thr. pro-
-asx. asx-	-glux. ala-	-phe. glux	-val. ala-
-asx. gly-	-glux. leu-		-val. asx-
			-val. cys-
			-val. glux-[35]

Ovalbumin: -ala. gly. val. asx. ala. ala-[36]

-asx. ser. glux. ileu. ala-

-glux. ser. ala-[37]

-cys. ala-	-thr. cys-	-cys. phe-
-cys. gly-	-val. cys-	-gly. cys-
-cys. val-	-cys. glux-	-asx. cys-[38]
-ser. cys-	-phe. cys-	
	-val. ser. pro.[28]	

Papain: Ileu. pro. glux-[39]
-ser. asx- -cys. asx- -val. cys-[40]
-asx. cys- -cys. gly. asx-

Hemoglobin: Val. glun. leu-
Val. leu-[41]
-lys. arg- -arg. leu- -val. lys-
-lys. leu- -phe. lys- -arg. lys-
-ser. arg- -arg. phe- -tyr. arg-
-ala. arg- -leu. arg- -phe. arg-[42]

Myoglobin: Gly. leu-[43, 44]

γ-Globulin: Ala. leu. val. asx. glux-[45, 46]

β-Lactoglobulin: -val. glux- -thr. lys- -ala. lys-
-val. leu- -lys. gly- -leu. lys-
-asx. lys- -pro. lys- -phe. lys-[47]
-glux. lys- -lys. pro-

Carboxypeptidase: Asn. ser-
-ser. thr-[48]

-thr[h]-

Serum albumin: Asx. ala[g]-[49]

Lactogenic hormone: Thr. pro. val. thr. pro-[50]

Tobacco mosaic virus: -thr. ser. gly. pro. ala. thr.[51]
Pro. ileu. glux-[52]

Casein: Lys. leu. val. ala. glux. asx-[53]
-leu. gly- -ser. pro- -ser. leu-[47]

Chymotrypsinogen: -gly. leu. ser. arg. ileu. val-[54, 55]
-tyr. thr. asn. ala-[56]
-gly. asp. ser. gly-[57]

SEQUENCES NOT USED IN THE GRID

Serum albumin: -gly. val. ala. leu[h].[58]
-ser. val. thr. leu. ala. ala[a].[58]

Actin: -his. ileu. phe.[59]

Tropomyosin: -ala. ileu. met. thr. ser. ileu.[59]

Pepsin: Leu. gly. asp. asp. his. glu-[60] (cf.[61])

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INFECTION OF PROTOPLASTS BY DISRUPTED T2 VIRUS

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INTRODUCTION

The functions of the components of virus particles have been the subject of much investigation in two widely different virus systems. In the case of tobacco mosaic virus (TMV) it has recently been demonstrated that the ribonucleic acid isolated from the virus retains infectivity[1, 2] and possesses the property of genetic determination.[3] In contrast with the low infectivity of TMV, bacteriophage T2 can be studied with very high precision. The protein coat of T2 isolated after osmotic rupture has been found to adsorb to the receptor site on the cell wall of the host and to stop multiplication of the host cell.[4] After injection through the tail of T2 virus into the host cell, the deoxyribonucleic acid (DNA) component is found to be associated with the replicating activity of the virus.[5] However, no direct biological activity of the isolated DNA has been demonstrated. It would be of much interest to determine whether the biological activity of the protein moiety subsequent to attachment[4, 6] is a necessary prerequisite for the function of the DNA portion or, indeed, whether DNA can be active in the complete absence of protein.

Since cell wall components and the protein tip of T2 virus interact prior to injection of DNA, it was considered possible that the removal of the cell wall might permit the replicating moiety of the virus to enter the cytoplasm directly. Removal of cell walls can be achieved by lysozyme treatment of *Escherichia coli* B in hypertonic sucrose to produce forms known as "protoplasts."[7, 8] Intact T2 virus will not infect these protoplasts. We have attempted to infect protoplasts directly with DNA isolated from T2, without success. However, when osmotically disrupted preparations of T2 virus[9] were mixed with protoplasts of *E. coli* B, significant increases in the numbers of infective particles were observed. In fact, similar increases in infective units were obtained with protoplasts of a number of bacteria whose cells are normally resistant to infection. Although the osmotically shocked preparations employed in these studies contained all the components of the original virus, we have obtained evidence to indicate that the activity observed may be due to DNA units protected by a protein shell. This communication describes some of the data obtained on the interaction of protoplasts and disrupted T2 virus, as they relate to the functional requirements of virus-cell interaction.

METHODS

Preparation of Protoplasts.—Protoplasts of *E. coli* B and other bacterial strains were prepared by a modification of a procedure suggested by Roberts,[7] similar to that of Zinder and Arndt.[8] The bacteria were grown in Difco nutrient broth for 3 hours at 37° C. on a reciprocal shaker, starting with 2×10^7 cells per milliliter. The cells were then harvested by centrifugation and resuspended in the same volume of 0.5 *M* sucrose and 0.01 *M* tris(hydroxymethyl)aminomethane at pH 9, containing 12.5 μg. lysozyme per milliliter. The mixture was incubated for 5 minutes at 37° C. and then centrifuged, and the sediment was resuspended in the same volume of 0.25 *M* sucrose containing nutrient broth at one-half concentration. The protoplast spheres so produced were enumerated in a Petroff-Hauser chamber, using the phase-contrast microscope. With *E. coli* B under the conditions described, an average of 4×10^8 spheres per milliliter were produced from 2×10^9 viable bacteria per milliliter.

Preparation of T2 Shockates.—Phage strain T2r+ was propagated on *E. coli* B in a glucose–ammonium salts medium[10] and concentrated by differential centrifugation in an SS-1 Sorvall centrifuge, first at $3{,}000 \times g$ to remove cellular debris and then at $20{,}000 \times g$ for 45 minutes. In the preparation of osmotically shocked suspensions, phage concentrates were diluted to 2×10^{11} plaque-forming particles per milliliter in 3*M* NaCl for 5 minutes at room temperature and rapidly diluted 40-fold in 1 per cent gelatin solution, resulting in 99 per cent or better reduction in infectivity. T2 shockates heated to 71° C. for 15 minutes or T2 phage suspensions heated to 71° C. for 15 minutes followed by osmotic shocking were also employed in these studies. The latter preparations will be referred to as "heated shockates."

"Infection" of Protoplasts with Heated Osmotic Shockates of T2.—It was found that heated preparations of T2 shockate which contain $<10^2$ infectious particles per milliliter as determined by plaque counting with whole cells produced infective T2 when incubated with protoplasts of *E. coli* B or *E. coli* B/2. T2 phage increases as high as 10^9 plaque-forming particles per milliliter were observed in 60 minutes at 37° C. Infective phage formation was not observed when heated shockates were mixed with protoplasts which were suspended in broth or water without sucrose.[11] Nor were any viable phage formed when suspensions of whole cells with or without sucrose were used. The possibility of some type of "multiplicity reactivation"[12] with intact cells was ruled out by using diluted suspensions of cells; in no instance was any viable T2 formed.

It was of some interest to investigate in detail the production of virus in protoplasts from a resistant strain, since reinfection with any intact virus of cells remaining after lysozyme treatment cannot occur. *E. coli* B/2 was used for these studies, and it was found that the general characteristics of viral formation were similar to those with *E. coli* B. Figure 1 illustrates the kinetics of phage formation in *E. coli* B/2. Aliquots of the mixture of protoplasts and heated T2 shockate were removed at intervals and plated with sensitive *E. coli* B. Although it is not certain that all "intraprotoplast" infective particles were thus being assayed, some idea of the formation of infective particles was obtained. Thus, within the first 5 minutes, we observe the appearance of 2×10^2 T2 phage per milliliter, which

may be due to disaggregation and release of intact T2, since in a number of preparations made in identical fashion we could show by dilution and incubation in the absence of protoplasts that approximately this quantity of intact virus was present. Nevertheless, it represents only a very small fraction of the phage subsequently formed. This is followed by a lag period up to 11 minutes, a period of rapid multiplication lasting a little over 20 minutes, and a period of diminished multiplication after 30 minutes, yielding 7×10^7 T2 units per milliliter.

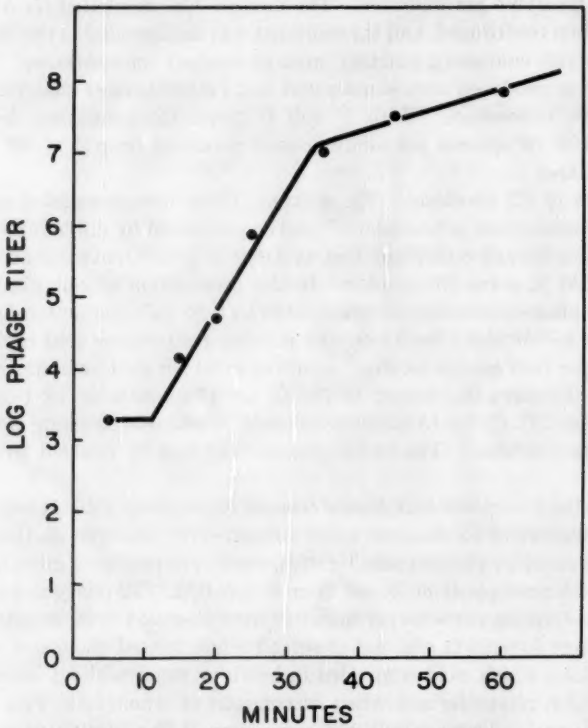

Fig. 1.—Formation of infectious T2 phage from heat-treated and osmotically shocked T2 (noninfectious preparation) in protoplasts of *E. coli* B/2 at 37° C.

No quantitative estimates of the rates of adsorption and the yields of virus per protoplast have as yet been made. Experiments were performed in which mixtures of protoplasts and heated shockate were centrifuged after incubation for 5 minutes at 37° C. and the protoplasts resuspended in 0.25 *M* sucrose and broth. The infective virus T2 produced after 60 minutes at 37° C. was then determined and found to be nearly the same as that formed in the uncentrifuged mixtures. This indicates that rapid adsorption of the disrupted virus to the protoplast occurs under these conditions.

Certain conditions have been found essential for infective T2 virus synthesis. Thus versene (0.01 *M*) completely abolished the activity, and Mg ions (0.001 *M*)

gave some stimulation. Sodium chloride at concentrations of 0.1 *M* or greater were found to inhibit. Virus formation was completely prevented in the absence of casein hydrolyzate or nutrient broth. Finally, certain metabolic poisons have been found to inhibit, namely, potassium cyanide, potassium arsenite, and potassium arsenate at 0.001 *M* concentration or higher.

Characteristics of Heat-treated T2 Shockate.—When heat-treated T2 shockate was incubated with crystalline trypsin (Nutritional Biochemicals; 50 μg/ml) for 30 minutes and soybean trypsin inhibitor (Worthington Biochemical Company; 1 mg/ml) added prior to mixing with protoplast suspensions, only 0.1 per cent as much of the infective T2 was produced as compared with that formed in the absence of trypsin treatment (Table 1). Crystalline desoxyribonuclease (DNAase) (Worthington Biochemical Company) and a highly purified pancreatic DNAase (kindly supplied by Dr. Werner Baumgarten, Merck and Company) destroyed less of the activity than did trypsin; however, a comparable destruction occurred in the presence of citrate, which would inhibit the enzymatic action on free desoxyribonucleate. The effect of DNAase on heated shockate may therefore be due to contaminating enzymes. Both trypsin and DNAase had little or no inhibitory action when added 10 minutes after protoplasts and heated shockate were mixed.

TABLE 1

INFECTIVITY OF HEATED T2 SHOCKATES ON PROTOPLASTS OF *E. coli* B

TREATMENT OF HEATED SHOCKATE*	+ *E. coli* B PROTOPLASTS† Initial Titer/Ml	+ *E. coli* B PROTOPLASTS† Infectivity Titer/Ml, 60 Min.
None	$<10^2$	8×10^7
50 μg. trypsin, 30 min., 37°, + 1 mg. antitrypsin	$<10^2$	2×10^4
50 μg. DNAase + 0.005 *M* Mg, 30 min., 37°, + 0.01 *M* citrate	$<10^2$	2×10^6
50 μg. DNAase + 0.01 *M* citrate, 30 min., 37°	$<10^2$	1×10^6
Versene, 0.01 *M*	$<10^2$	$<10^2$
Homogenized in Waring Blendor or Mickle agitator, 2 min.	$<10^2$	$<10^2$
Ultraviolet, 1-mm. layer, 30 inches from GE 15-watt lamp, 5 sec.	$<10^2$	$<10^2$

* Heated shockate: T2 virus at 10^{12} particles per milliliter, heated to 71° C., 15 minutes; dissolved in 3 *M* NaCl, 5 minutes, and rapidly diluted 1:40 in 1 per cent gelatin. Infectivity titer, $<10^2$ plaque-formers per milliliter.

† Protoplasts: 3-hour broth growth of *E. coli* B, treated with 12.5 μg/ml lysozyme in 0.5 *M* sucrose, centrifuged and resuspended in 0.25 *M* sucrose-broth.

The activity of heated shockate was completely inhibited by homogenization for brief intervals in a chilled Waring Blendor or by agitation in the Mickle disintegrator (Table 1). In addition, complete inactivation was effected by a 5-second exposure in a 1-mm. layer to a GE 15-watt germicidal ultraviolet lamp at 30 inches, under conditions where little T2 phage inactivation could be observed.

These data indicate that "heated shockate" contains disrupted viral particles capable of inducing virus synthesis in the presence of protoplast suspensions. The sensitivity of these disrupted viral forms to the action of trypsin contrasts with the resistance of normal viral particles. That the nucleic acid of the subparticles which participate in viral synthesis is probably in a more exposed form than in intact virus T2 is indicated by the extreme lability to ultraviolet and homogenization. It is tempting to visualize that in the process of osmotic disruption and heat treatment of T2, packets of DNA are produced which are kept intact by the protein

layer of the head membrane, which serves to keep the DNA from forming copolymers. This is further suggested by the fact that in experiments with DNA (at 1 mg/ml) isolated from T2 virus (using the phenol extraction procedure of Gierer and Schramm[13] and the acetate-duponol method of Mayers and Spizizen[14]), it was found that no phage synthesis occurred on mixing with protoplasts. Discrete packages of DNA may then be required for infection of protoplasts, since DNA has been clearly shown to be required for T2 phage synthesis.[5]

Protoplast "Infection" with Unheated Osmotic Shockates of T2.—Phage increases have also been observed when an unheated osmotic shockate of T2 was mixed with protoplasts derived from *E. coli* B. Table 2 shows data from an experiment in which, after incubation at 37° C. for 60 minutes, an increase of 7×10^7 infective units per milliliter was observed. When citrate was added to DNAase-treated T2 shockate or soybean trypsin inhibitor to trypsin-treated shockate prior to mixing with protoplasts, no inhibition of virus formation occurred, unlike the effects produced with heated shockate. The addition of crystalline desoxyribonuclease, crystalline ribonuclease, or crystalline trypsin to the mixture of shockate and protoplasts completely inhibited the increase in infective particles. Thus the effects of the enzymes are probably on protoplasts (cf. Spiegelman)[15] and in this respect differ from intact-cell experiments in which virus growth is unaffected by these enzymes. T2 virus which was not shocked but was diluted to the same infectivity titer present in shockate did not multiply in the presence of protoplasts.

Centrifugation experiments with T2 shockate using the Spinco ultracentrifuge (Table 2) showed that the supernatant fluid, after centrifugation at 50,000 × *g*

TABLE 2

INFECTION OF *E. coli* B PROTOPLASTS WITH T2 OSMOTIC SHOCKATES (UNHEATED)

TREATMENT OF OSMOTIC SHOCKATE*	+ *E. coli* B PROTOPLASTS†	
	Initial Infectivity Titer/Ml	Increase in Infectivity/Ml, 60 Min.
None	5×10^6	7×10^7
DNAse, 100 μg/ml, + 0.005 *M* $MgSO_4$, 15 min., 37°	5×10^6	0
DNAase, 100 μg/ml, + 0.01 *M* citrate	5×10^6	7×10^7
RNAase, 100 μg/ml, 15 min., 37°	5×10^6	0
Trypsin, 100 μg/ml, 15 min., 37°	5×10^6	0
Trypsin, 100 μg/ml, 15 min., 37°, + 1 mg. antitrypsin	5×10^6	7×10^7
Supernatant of Spinco centrifugation, 50,000 × *g*, 60 min.	2×10^5	4×10^6
Phage control, diluted in 1 per cent gelatin	5×10^6	0

* Osmotic shockate: T2 virus at 2×10^{11} particles per milliliter in 3 *M* NaCl for 5 minutes at room temperature; rapidly diluted 40-fold in 1 per cent gelatin.

† Protoplasts: 3-hour broth growth of *E. coli* B, treated with 12.5 μg/ml lysozyme in 0.5 *M* sucrose, centrifuged and resuspended in 0.25 *M* sucrose-broth.

for 60 minutes, retained only a fraction of its original activity. Thus the supernate contained 4 per cent of the infectious virus, and the increase in T2 on incubation with protoplasts was only 5.8 per cent of that obtained with the uncentrifuged shockate. The bulk of the activity could be found in the sediment. Centrifugation of shockate at 20,000 × *g* for 30 minutes, however, did not sediment the bulk of the activity observed with protoplasts, although more than 90 per cent of the intact virus was thus sedimented. Thus the activity with protoplasts is probably due to disrupted particles with similar but not identical sedimentation behavior to intact phage.

Virus T2 Formation in Protoplasts of "Resistant" Bacteria.—Of much interest was the observation that disrupted T2 viral preparations could produce infectious virus in protoplasts from bacterial species which are resistant to viral infection. Table 3 summarizes the results.[16] The resistance of those cells in the intact state

TABLE 3

PHAGE T2 PRODUCTION FROM INACTIVATED SHOCKATE IN PROTOPLASTS OF DIFFERENT BACTERIAL SPECIES

Organism	Sensitivity of Intact Cells to Infective T2 Phage (10^6/Ml)	Phage T2 Formed/Ml in 60 Min. from Inactivated T2 Shockate* in Protoplasts†
E. coli B	+	(>) 10^9
E. coli B/2	0	(>) 10^8
E. coli K12	+	5×10^3
E. coli Crookes	+	1.5×10^5
Anaerogenic *E. coli* (WR-2)	0	10^4
Atypical *Klebsiella* (WR-3)	0	5×10^3
Providence group paracolon (WR-6)	0	1.3×10^4
A. aerogenes 417	0	2×10^6
Proteus vulgaris (WR-1)	0	0
B. megaterium 899	0	0
Pseudomonas saccharophila	0	0

* T2 heated to 71°, 15 minutes, and shocked; infectivity for *E.* coli B intact cells, $< 10^2$ plaque-forming particles per milliliter.

† Protoplasts prepared from 3-hour growths in nutrient broth, as described in text.

whose protoplasts were found to produce virus is apparently due to lack of proper receptor sites in the cell walls. We have also found a number of resistant organisms whose protoplasts will not support virus T2 synthesis from heated shockate, namely, the *Proteus*, *Bacillus megaterium*, and *Pseudomonas saccharophila* strains tested. The nature of the resistance of the latter organisms remains to be determined.

DISCUSSION

Multiplication of bacteriophage was observed by other investigators in protoplasts of *B. megaterium*,[17, 18] *B. subtilis*,[19] and *E. coli*,[8] provided that infection with virus was performed prior to removal of surface components containing the specific receptors for viral adsorption. Direct infection of protoplasts with intact virus has not been observed.

The present experiments show that disrupted particles of T2 virus can produce infectious virus in protoplasts from sensitive and certain resistant strains of bacteria. By the removal of cell wall components, it is possible to determine whether the protoplasm is or is not able to support viral propagation. Thus *Aerobacter aerogenes* 417 is highly susceptible in its protoplast state to coliphage T2 shockates, whereas the intact cells are completely resistant to infection with T2 virus. The specific cell receptor–viral tail combination is thus replaced by a less exacting protoplast-disrupted viral interaction.

Two types of disrupted T2 preparations have been used in these studies of protoplast infection. Unheated osmotic shockate of T2 is unaffected by trypsin or desoxyribonuclease. The heated osmotic shockate apparently contains more degraded forms of T2 virus, since trypsin almost completely destroys its ability to infect protoplasts. This indicates the requirement of some protein for the production of T2 phage in protoplasts. This latter protein may be part of an envelope, protecting the DNA from forming large polymers. The formation of large molecu-

lar aggregates of DNA, as occurs during its isolation free of protein, may prevent its entrance into protoplasts. On the other hand, the protien may be involved in attachment to the protoplast membrane or may actually participate in the formation of viral particles.

Intact protoplastic structure appears to be essential for phage production, since "shocking" of protoplasts by removal of hypertonic sucrose prevents the formation of infective virus. The requirement for amino acids and the inhibitory action of metabolic poisons suggest that complex biochemical events are probably involved in viral formation from shockate in protoplasts.

The data presented do not establish definitely that new viral particles were being synthesized by some form of replication. In most experiments, in fact, less virus was recovered than was originally employed for the preparation of disrupted viral particles. In certain experiments, however, it has been possible to recover more infective T2 after incubation of heated T2 shockate with protoplasts. This was also found to be the case when protoplasts of resistant strains were employed, where infection of whole cells with T2 virus could not account for any of the increases observed. Nevertheless, the possibility must be considered that some form of reactivation might have occurred, involving replacement or synthesis of some portion of the virus, which then makes it possible to infect intact sensitive cells. It is also possible that some type of multiplicity reactivation may have taken place in the protoplasts, involving the participation of a number of damaged particles. Since phage multiplication is known to occur in protoplasts, our results may be accounted for by viral reactivation within protoplasts with subsequent replication prior to liberation. Studies are in progress to elucidate this problem.

Thus the use of protoplasts provides us with a technique for the study of the "minimal unit" capable of viral formation.

SUMMARY

Disrupted T2 virus preparations possessing no measurable infectivity have been found to produce infectious T2 virus after incubation with protoplasts from *E. coli* B or a number of other bacteria normally resistant to T2 infection. Virus production occurs only in intact protoplasts, that is, in hypertonic sucrose, and requires amino acids. Although protein-free DNA isolated from T2 virus cannot produce infective virus, evidence is presented that DNA units protected by a protein shell may be involved in the production of T2 virus in protoplasts.

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INCORPORATION OF RADIOACTIVE CARBON INTO A PTERIDINE OF A BLUE-GREEN ALGA

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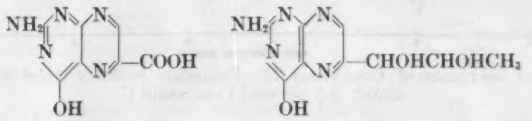
In a preliminary paper[1] we have reported a striking relationship between the loss of photosynthetic activity in a blue-green alga, *Anacystis nidulans*, and the release from the algal cells of a group of closely related pteridines. Two of these compounds make up the greater bulk of the fluorescent materials released; one is a yellow crystalline compound which was described briefly in our previous paper.[1] The second is a blue fluorescent compound (hereinafter called "Compound C") which appears to be the principal material obtained from the cells when they are killed with acid.

Compound C has been obtained chromatographically pure in small quantities, and some observations have been made on its chemistry, although these have necessarily been limited by the quantities so far available. In the first place, it is readily oxidized by alkaline permanganate to 2-amino-4-hydroxy-6-carboxypteridine (*I* in the accompanying diagram), as previously reported, and it has ultra-



I II

violet and infrared absorption spectra (Figs. 1 and 2) very similar to that of 2-amino-4-hydroxy-6-(1′,2′-dihydroxypropyl) pteridine (biopterin; *II* in the diagram).[2, 3] It is readily distinguished from the latter compound, however, by its Rf values in various chromatographic solvents (Table 1). Compound C is quite stable to alkali, being recovered unchanged after 2 hours of heating with 2 *N* sodium hydroxide at 100° C. On the other hand, when treated with 2 *N* hydrochloric acid under the same conditions, it is degraded quantitatively to a different fluorescent substance which proved to be identical, paper-chromatographically and spectrophotometrically (ultraviolet and infrared), with biopterin. These

facts, then, suggest that Compound C contains an aldehyde linked in an acetal grouping across the vicinal hydroxyl groups of biopterin. So far, we have not been

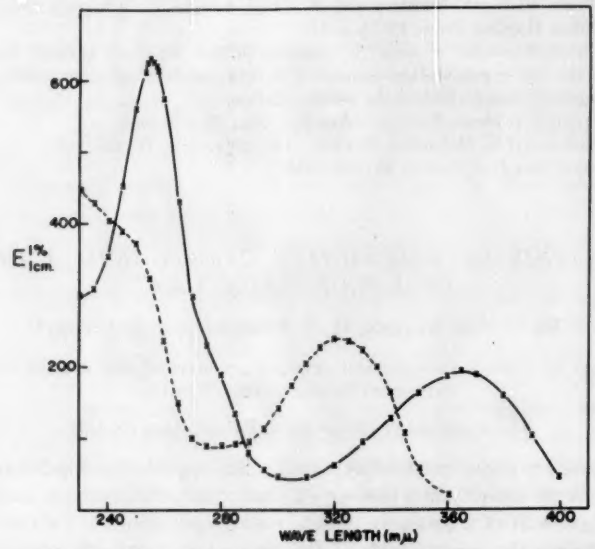


FIG. 1.—Ultraviolet absorption spectrum of Compound C: × — × — × = in 0.1 *N* sodium hydroxide; × - - - × - - - × = in 0.1 *N* hydrochloric acid.

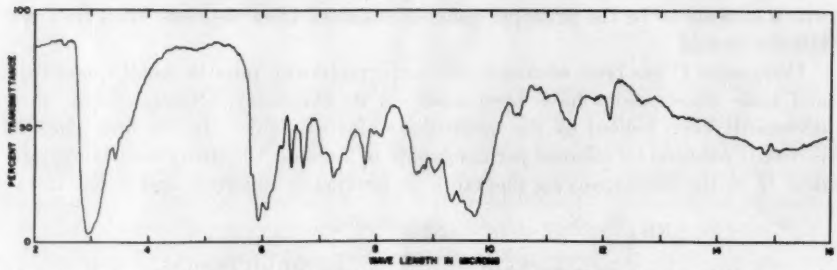


FIG. 2.—Infrared spectrum of Compound C. Potassium bromide wafer containing approximately 0.5 per cent Compound C.

able to identify the fragment which is released in this hydrolysis. It appears to be a low-molecular-weight compound which is not acidic (since Compound C is virtually neutral, as shown by paper electrophoresis).

Although the structure of Compound C is not yet fully defined, we wish to report a series of experiments in which it has been shown that, over a short period of time,

TABLE 1

Solvent	Biopterin	Compound C	Solvent	Biopterin	Compound C
n-Butanol, acetic acid, water (4:1:1)	0.31	0.10	4 per cent sodium citrate	0.60	0.73
n-Propanol, 1 per cent ammonia (2:1)	0.46	0.37	*sec*-Butanol, formic acid, water (8:2:5)	0.64	0.47

radioactive C^{14} as $C^{14}O_2$ is incorporated by *A. nidulans* into the acid hydrolyzable fraction of Compound C but not into the 1′,2′-dihydroxypropyl side chain.

Experimental.—Freshly harvested cells grown in a continuous culture chamber[4] were washed and suspended in phosphate buffer (pH 7.5; 0.02 *M*). Eighteen milliliters of cell suspension containing approximately 3 mm^3/ml of cells were placed in a special, flat Warburg vessel (total volume, 60 ml.; area, 57 $cm.^2$), fitted with a Siamese side arm. Approximately 2 mg. of $BaC^{14}O_3$ (0.36 mc/mg) were placed in one of the side arms and 0.5 ml. of 10 per cent hydrochloric acid in the other. The vessel was uniformly illuminated by a neon grid of 4,100 $\mu watts/cm^2$ (<7,000 A). The cell suspension was shaken for 5 minutes in the light, and the $C^{14}O_2$ was then released in the side arm. After a further 5 minutes of shaking in the light, the cells were killed by adding the remaining acid from the side arms. To insure killing and release of the pteridines, the flask was heated for 2 minutes at 100° C., after which time the contents were filtered through Celite, to give a colorless, acidic extract.

The fluorescent materials in this extract, previously adjusted to pH 4, were adsorbed on a Magnesol[5] column (1 × 15 cm.), which was then washed very thoroughly with water, followed by 5 per cent aqueous acetone. At this point fluorescent materials were beginning to appear in the eluate, and elution of these was then completed with 30 per cent aqueous acetone. Combined fractions containing Compound C were evaporated to small bulk, and the concentrated solution was applied to a single spot on a sheet (46 × 56 cm.) of Whatman No. 3 mm. filter paper. The chromatogram was developed in the first direction with *sec*-butanol, formic acid, water (8:2:5) and in the second direction with *n*-propanol, 1 per cent ammonia (2:1). The fluorescent material corresponding to Compound C was eluted from the paper with water and rechromatographed against authentic Compound C in three more solvents, its radioactivity and quantity being measured after each chromatogram (see Table 2). The quantitative retention of radioactivity with fluorescence after each step is taken as convincing evidence of the purity of the radioactive Compound C.

TABLE 2

MEASUREMENT OF COMPOUND C AND OF ITS RADIOACTIVITY*

Sample	Solvent	Total Counts/Min. Recovered Corrected for Background	Recovered Compound C Measured Fluorometrically (μg.)†
Light	*sec*-Butanol, formic acid, water (8:2:5)	161	5.10
	n-Propanol, 1 per cent ammonia (2:1)	156	5.10
	n-Butanol, acetic acid, water (4:1:1)	112	3.56
Biopterin recovered after hydrolysis	*n*-Butanol-acetic acid, water (4:1:1)	3	2.00
Dark	*sec*-Butanol, formic acid, water (8:2:5) and n-Propanol, 1 per cent ammonia (2:1)	0	3.08

* All chromatograms were ascending, on Whatman No. 3 mm. Fluorescence measurements were done with a Farrand Fluorometer Model A. Radioactivity was determined with a Tracerlab windowless flow counter, SC-16, and a Tracerlab Utility Scaler.
† Estimated against a weighed standard of Compound C.

After the final chromatogram, Compound C was hydrolyzed in 2 *N* hydrochloric acid (0.2 ml.) for 30 minutes at 100°, and, after removal of the acid over potassium

hydroxide in a desiccator, the residue was chromatographed against authentic biopterin. As shown in Table 2, the recovered biopterin was not radioactive.

This experiment has been carried out three times in all, using various different procedures for the purification of the Compound C (e.g., Filtrol[6] columns, instead of Magnesol, used before or after paper chromatography; different solvent combinations for paper chromatography, including the use of dilute acetic acid as an alternate solvent); the results in each experiment were the same as those recorded above.

When an exactly similar experiment was carried out with cells incubated in the dark, no radioactivity was found in the Compound C recovered (Table 2).

Discussion.—From the work of our previous paper[1] and that reported herein, the following facts may be marshaled. From *A. nidulans* and several other blue-green algae a number of pteridine compounds can be extracted in quantities of about 0.1 per cent of the cell dry weight. Variation in distribution of the various pteridines with the method of extraction suggests that they result from one or two parent compounds in the cells. Concomitant loss of pteridines and photosynthetic activity during incubation of *Anacystis* at 4° C. suggests a role in photosynthetic metabolism.

Nathan, Hutner, and Levin[7] recently have shown for the parasitic trypanosomid *Crithidia fasiculata* a growth-factor requirement which can be supplied by biopterin independently of folic acid. It was postulated that "Crithidia factor," an unconjugated pteridine, identified with biopterin, provides for a metabolic function other than that performed by folic acid. Our data raise the question whether biopterin itself is the metabolically active compound in the cells. No significant quantity of biopterin is extractable from *Anacystis*, although at least one of the extractable pteridines is readily converted to biopterin by acid hydrolysis. On the other hand, our data provide direct evidence for the postulate that compounds of the biopterin type are active metabolically in a role different from those of the folic acid group.

Of the several pteridines extracted from *Anacystis*, one, Compound C, is converted by acid hydrolysis to biopterin and a fragment which has not yet been identified. Since the ultraviolet and infrared spectra of biopterin and Compound C are almost identical, the unidentified fragment must be attached to the side chain of biopterin and must be relatively inert spectrophotometrically. During photosynthetic metabolism of *Anacystis*, C^{14} from $C^{14}O_2$ is rapidly incorporated into the unidentified fragment of Compound C but not into the biopterin portion (cf. Table 2).

Since the identity of the C^{14}-labeled fragment of Compound C is unknown, there remains the possibility that the recovered radioactivity is due to an impurity which carries through all the chromatographic separations and finally separates chromatographically only after acid hydrolysis. We consider this possibility highly unlikely in view of the strict correspondence between the radioactivity and measured fluorescence of Compound C in successive chromatograms.

The metabolic locus of action of Compound C (or its cellular parent compound) is not specified by the evidence at hand. Labeled carbon is incorporated into the unidentified fragment only in the light. However, *Anacystis* is an obligate photoautotroph with a very low rate of endogenous (dark) metabolism. The question whether the locus of action is associated with the early stages of carbon dioxide

fixation and reduction is not answered by the evidence presently available. A cautious hypothesis must be limited to this statement: A pteridine related to biopterin acts as a metabolic carrier for a carbon fragment.

Work is in progress on the complete identification of Compound C and its relation to the other pteridines previously described in *Anacystis*. Such information should allow a more precise statement of the metabolic role of these compounds.

Summary.—A labeled pteridine has been obtained from *A. nidulans* during photosynthesis in the presence of $C^{14}O_2$. After acid hydrolysis, this pteridine yielded unlabeled biopterin and a labeled and as yet unidentified fragment. The possible metabolic significance of this and related compounds is discussed.

* This work was supported by the Rockefeller Foundation and by the Robert A. Welch Foundation, Houston, Texas.

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*IONIC SEMIPERMEABILITY AS A BULK PROPERTY**

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One of the most fundamental facts of biology is the unequal distribution of K^+ and Na^+ in and outside the cell, K^+ being found in a relatively high concentration inside and Na^+ outside. This unequal distribution is attributed to the function of the cell membrane, to its selective permeability and "pumping action." No generally accepted picture has yet been developed for the mechanism of this hypothetical activity of the membrane, and so it seems justifiable to consider alternate possibilities.

The water molecule has such a distribution of charges, including lone pairs of electrons, as to lend it a tendency to link molecule to molecule in a tetrahedral arrangement. In ice this regular association extends throughout the whole structure. In liquid water the structure is broken up by heat agitation into small fragments, and there is no long-range regularity. The fragments of the crystalline structure are very small and have a temporary existence, associations being constantly formed and broken.

There is strong evidence that around surfaces the small crystallites may join to more extensive lattices, establishing a long-range regularity reaching many hundreds of molecules deep into the fluid.[1] So it is not impossible that most of the water within densely packed biological structure is in a more or less ordered state.

While in K^+ the nuclear charge is screened by three electron shells, in Na^+ it is screened only by two. Moreover, the dimensions of K^+ are very similar to those of the water molecule, which is not the case with Na^+. Consequently we can expect that K^+ does not disturb the regularity of the water structure, while Na^+ does and, by ordering the water dipoles around itself, creates disorder in the water structure; K^+, on the other hand, may readily be substituted for water molecules. This difference is borne out by the temperature dependence of the conductivity of NaCl and KCl as well as by the "B factor" of both cations.[2] When Na^+ is introduced into lattice-ordered water structure, bonds have to be broken which hold the water molecules together, and work will have to be done. Correspondingly, we can also expect the system to tend to eject Na^+ already introduced and to balance its negative charges with preference by K^+. This allows us to construct a theory which picture the ionic semipermeability of cells as a bulk property, while earlier theories ascribed semipermeability to the "membrane" only.

Such a theory suggests experiments and calculations along different lines. First, we can attempt to demonstrate the deranging activity of Na^+ and the inactivity of K^+. Second, we can try to demonstrate actually the preference of biological structures for K^+ in the absence of a membrane. Third, we can calculate whether the dipole forces of water are sufficient to account for the known distribution of K^+ and Na^+ in biological material.

It has been suggested by one of us[1] that the phosphorescent behavior of various fluorescent substances in a frozen watery solution depends on the orderly structure of water and its interaction with the excited molecule. It has also been shown that Na^+ causes a strong disturbance in the light emission of Rhodamin B, while K^+ does not do so. Equally inactive is NH_4^+, the physical properties and dimensions of which are very similar to those of K^+, while Li^+ was found to create even graver disturbance than Na^+. The reaction with Rhodamin was too complex to allow a simple interpretation. The situation is simpler with tryptophan, adenosine triphosphate, and nucleic acid. All these substances are excited to triplets[3] and show in the presence of 0.25 per cent glucose a long-lived phosphorescence if their watery solution is frozen in dry ice and illuminated by ultraviolet light. If their triplet excitations are stabilized by the water structures, then the afterglow must be quenched by any substance which interferes with the regularity of these structures, and we have to expect Na^+ to cause quenching, while K^+ is inactive. The experiment showed that KCl, in physiological concentrations such as 0.1 M, had no effect on the lifetime of the triplet excitation of the above systems and did not materially reduce the duration of the afterglow, while NaCl, in the same concentration, had a strong quenching action.

For the demonstration of the preference of cellular structures for K^+ in the absence of an active membrane, we used glycerinated muscle fibers. According to our experience, glycerination destroys the "membrane," as indicated by the complete absence of electric excitability and conduction. Moreover, in the presence of the high K^+ concentrations employed, the membrane, even if present, could be expected to be inoperative. So we tried to decide in the experiment whether such a glycerinated material is capable of distinguishing between K^+ and Na^+. The difficulty of the experiment lies in the fact that we can expect undisturbed water structures in resting muscle only, and the charge distribution in this material must be greatly in-

fluenced by the tetravalent ATP anions present. However, it is very difficult to produce a glycerinated muscle which is completely relaxed in the presence of ATP. To do this, the so-called "relaxing factors" have to be present and the glycerol treatment has to be of short duration. The objection may be raised that in such a carefully treated material the "membrane" may not completely be destroyed.

Our experiments were performed in the following manner: 2–3 mm. thick bundles of the psoas muscle of freshly killed rabbits were tied to applicator sticks and immersed for 3–6 hours in 50 per cent glycerol at 0° C. Then the muscle fascicles were transferred into a muscle extract prepared according to Bendall.[4] The muscle fascicles remained in this extract for another 2 hours, in order to provide them with a sufficient amount of relaxing factor to prevent their contraction in the next bath, which consisted of the same extract diluted with an equal volume of salt solution. Here the final concentration of KCl and NaCl was 0.08 *M*, that of $MgCl_2$ was 0.004 *M*, and that of ATP was 4 m*M*. After 1-hour incubation at room temperature the muscle was decomposed into 0.1–0.2-mm. thin fiber bundles which were left in this liquid for another 20–40 minutes. After this time a number of fiber bundles (20–40) were liberated from adhering fluid by placing them on filter paper, then dissolved in concentrated nitric acid and analyzed for K and Na on the flame photometer. The results are summarized in Table 1. As the table shows, no significant difference was found between the relative concentrations of K and Na in the bath and in the fibers.

TABLE 1

Experiment No.	K/Na Ratio in Bath	K/Na Ratio in Muscle
I	0.96	0.98
II	1.09	1.14 1.12
III	1.12	1.10

As is well known, Ca plays a dominant role in muscle function and ionic distribution. In order to maintain its normal ionic K/Na balance, the muscle must be provided with Ca^{2+}. For this reason, the above experiments were completed by adding $CaCl_2$ in 0.5 m*M* final concentration to the bath. In the first experiment the intracellular K/Na ratio rose within 5 minutes from 1.07 to 1.31. In the second experiment the quotient rose within 3 minutes from 1.07 to 1.33. After 4 minutes it went up to 1.8. In the third experiment the quotient rose within 4 minutes from 1.10 to 1.52 and within 9 minutes to 1.77. In a fourth experiment the fibers were immediately placed in a Ca-containing bath; the K/Na ratio was found within 4 minutes to be 1.30, in 12 minutes, 1.35, while in the bath it was 1.05.

On more prolonged incubation the quotient dropped again, which may have been due to the disorganizing effect of the contracture induced by the addition of Ca. It seems that two effects were counteracting one another: the Ca tended to increase the quotient of K/Na, while the developing contraction tended to equalize the ionic distribution. It is unfortunate that no relaxed state could be maintained in the presence of Ca.

The experiment is a rather imperfect one, and the quotients obtained are still far from the physiological quotient 10; nevertheless, the experiment definitely shows an unequal distribution, a trend toward eliminating Na^+ and retaining K^+.

The results discussed suggested an attempt to calculate the free energy of the ordering of water molecules. As is well known, the membrane potential of cells is about 0.1 volt. Therefore, the question arises whether the free energy of the ordering of water molecules is equal to or somewhat greater than 0.1 electron volt, which free energy would be necessary, according to the proposed mechanism, to produce the unequal ionic distribution found in cells.

Quantitative treatment of the ordering aspects of molecules in condensed phases is not easy. Whereas the distances between the molecules are largely determined by attractive and repulsive central forces, orientational forces also play an important role for the ordering of the molecules. To obtain an estimate of the free energy due to the orientational forces, we will consider only the interaction of nearest neighbors, for which we take only dipole-dipole interaction. Recently, Pople[5] has studied, on the basis of statistical mechanics, systems with noncentral force fields and has been able to derive an expression for the orientational free energy. His expression reduces, for the case of point-dipole interaction, to

$$F^{(or)} = -\frac{1}{6}\frac{z}{kT}\left(\frac{\mu^2}{a^3}\right)^2,$$

where $F^{(or)}$ represents the orientational free energy per molecule, z the number of nearest neighbors of a molecule, μ the dipole moment of a molecule, a the nearest-neighbor distance, T the absolute temperature, and k the Boltzmann constant.

Since the water molecules tend to arrange the other water molecules around themselves in a tetrahedron, we will take $z = 4$. The value of a in water is 2.9 A (Morgan and Warren[6]). Substituting these values together with $\mu = 1.85 \times 10^{-18}$ esu (Sänger and Steiger[7]) and the value of k, we find, at $T = 300°$ $F^{(or)} = -0.193$ electron volt (which, of course, is of the order of magnitude of the energy of the H-bond). It therefore seems that there is enough energy already in the locally ordered structures of molecules in liquid water, even taking into account only orientational forces and dipole-dipole interactions, to sustain electrical potentials of the order of 0.1 volt. If the order were more extensive than in liquid water, the energy would certainly be enough to account for the order of magnitude of bioelectrical potentials.

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*THE EFFECT OF GIBBERELLIN UPON FLOWER FORMATION**

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As reported in a preliminary note,[1] gibberellin was found to induce flower formation in a cold-requiring plant, a biennial variety of *Hyoscyamus niger* (henbane). This result was of interest in two respects: Gibberellin had been known only to promote shoot growth by cell elongation but not to have any effect on the initiation of flowers or other plant organs; chemical induction of flower formation has been largely an unsolved problem in plant physiology.

The studies on flower induction by gibberellin have since been extended to a number of other plants. The purpose of this paper is to summarize the results. In the meantime, this line of investigation has been taken up by other authors, too, and a definite picture is beginning to emerge about the effect of gibberellins in flower formation.

MATERIAL AND METHODS

Plants were chosen for this study in which flower formation is controlled either by low temperature or by photoperiod and which therefore can be maintained in the vegetative condition at will. Following is a list of the species and varieties that were used.

a) Biennials.—Plants with an obligatory cold requirement in flower formation: *Hyoscyamus niger* L. (henbane), two biennial strains; *Daucus carota* L. (carrot), varieties Early French Forcing and Danver's Half Long; *Petrosilenum crispum* (Mill.) Mansf. (parsley), varieties Hamburg and Plain; and *Brassica napus* L. (turnip), variety Purple Top Globe.

b) Winter Annuals.—Flower formation hastened by cold treatment but may ultimately take place without it: *Secale cereale* L. (Petkus winter rye).

c) Long-Day Plants.—*Hyoscyamus niger* L. (henbane), an annual variety; *Crepis tectorum* L.; *Samolus parviflorus* Raf. (water pimpernel); *Silene armeria* (garden or Sweet William catchfly); *Secale cereale* L. (Petkus spring rye). *Hyoscyamus, Crepis, Samolus,* and *Silene,* when grown on short day, remain vegetative for indefinite periods of time; spring rye initiates flowers on long and short day, but in the latter case with great delay.

d) Short-Day Plants.—*Glycine max* (L.) Merr. (soybean), variety Biloxi; *Xanthium pensylvanicum* Wallr. (cocklebur).

The plants were grown and treated in the greenhouse under strictly nonflowering (noninductive) conditions. Cold-requiring plants were kept at temperatures above 17° C., long-day plants were kept on a 9-hour day (light, 8:00 A.M. to 5:00 P.M.), and short-day plants were kept on photoperiods of at least 19 hours (supplementary light of approximately 30 foot-candles from 5:00 P.M. to 2:00 A.M.).

Prior to the start of the gibberellin treatment, the plants were allowed to reach an age at which they were fully capable of responding to thermo- or photoinduction.

Two types of gibberellin preparations were used. One, supplied by Dr. F. H. Stodola (USDA Northern Utilization Research Branch, Peoria, Ill.), consisted of

a mixture of gibberellin A_1 (= gibberellin A) and gibberellin A_3 (= gibberellin X = gibberellic acid). The other, received from Dr. C. Leben (Lilly Research Laboratories, Indianapolis, Ind.), contained only gibberellin A_3 and a small admixture of an unknown material which apparently has no gibberellin activity. The preparations were applied in aqueous solution, to which a wetting agent, Polyglycol-31 (Dow Chemical Company), had been added at a rate of approximately 0.5 ml. per 1-liter solution. Controls were treated with water *plus* wetting agent. Rye was treated either by administering the solution (0.1 ml.) into the angle between the youngest fully emerged leaf blade and the following leaf sheath or the stem, or by cutting the tip of one of the younger leaves and inserting the stump into a small vial with gibberellin solution. The second method gave a much greater response than the first. In the other species, 0.1 or 0.2 ml. of solution was applied once daily to the growing region of the shoot. The treatment was continued either for a specified period of time or until flower buds became visible. Plants that showed no visible flowering response by the end of the treatment were dissected (usually several weeks later) and examined for presence or absence of microscopic floral structures.

EXPERIMENTAL

Results with Cold-requiring Plants.—The results of gibberellin treatment of biennial plants are given in Table 1.[2] The experiments were conducted under long-

TABLE 1

EFFECT OF GIBBERELLIN TREATMENT ON FLOWER FORMATION IN BIENNIAL PLANTS

GIBBERELLIN (MICROGRAMS/PLANT/DAY)		*Hyoscyamus niger* (BIENNIAL) Purple-flowered Strain	*Hyoscyamus niger* (BIENNIAL) Yellow-flowered Strain	*Daucus carota* Early French Forcing	*Daucus carota* Danver's Half Long	*Brassica napus* PURPLE TOP GLOBE	*Petrosilenum sativum* Hamburg	*Petrosilenum sativum* Plain
0	No. plants treated	16	15	14	10	14	5	5
	No. plants flowering	0	0	0	0	0	0	0
	Weeks to anthesis	...	...	...	...	...	...	...
1	No. plants treated	28	...	9	10	14	...	...
	No. plants flowering	25	...	0	0	0	...	...
	Weeks to anthesis	8 to 12	...	...	...	...	...	...
2	No. plants treated	27	17	9	10	14	...	...
	No. plants flowering	26	17	3*	0	0	...	...
	Weeks to anthesis	8 to 12	5 to 8	>27	...	...	...	...
5	No. plants treated	...	...	14	10	14	5	4
	No. plants flowering	...	...	14*	7*	4*	0	1*
	Weeks to anthesis	...	...	18 to >27	>27	>27	...	>27
10	No. plants treated	60	18	14	10	14	5	5
	No. plants flowering	60	13†	14	10*	9*	0	0
	Weeks to anthesis	7 to 9	5 to 7	9 to 25	23 to >27	>27	...	...
20	No. plants treated	...	...	10	9	9	5	5
	No. plants flowering	...	...	10*	9*	7*	1*	2*
	Weeks to anthesis	...	...	20 to >27	>27	>27	>27	>27
50	No. plants treated	...	...	4	5	5	4	5
	No. plants flowering	...	...	3*	4*	3*	1*	0
	Weeks to anthesis	...	...	21 to >27	26 to >27	>27	>27	...
100	No. plants treated	...	...	4	5	5	5	5
	No. plants flowering	...	...	*	4*	3*	0	1*
	Weeks to anthesis	...	...	21 to >27	18 to >27	>27	...	>27

* Plants, or part of plants, failed to reach anthesis before end of experiment.
† Tip injured in plants that failed to respond.

day conditions. Since biennial plants, as a rule, require not only a cold treatment in order to form flowers, but also long photoperiods (which must be given following

the cold treatment), only one of the two blocks to flower formation which may be created in these plants was present in the experiments.

All four biennial species responded to gibberellin application with flower formation; the controls remained strictly vegetative. Optimal response was reached (except, perhaps, in parsley) with a daily dose of 10 μg. of gibberellin. Higher doses seem to be somewhat less effective; this point, however, needs confirmation.

The degree of response varied considerably, depending on the species. *Hyoscyamus* responded promptly and uniformly, carrot somewhat more slowly and less uniformly, the onset of anthesis extending over many weeks. In both species the gibberellin-induced flowering response was quite similar to the cold-induced one (Fig. 1). The flowers were normally developed and, at least in *Hyoscyamus*, fully

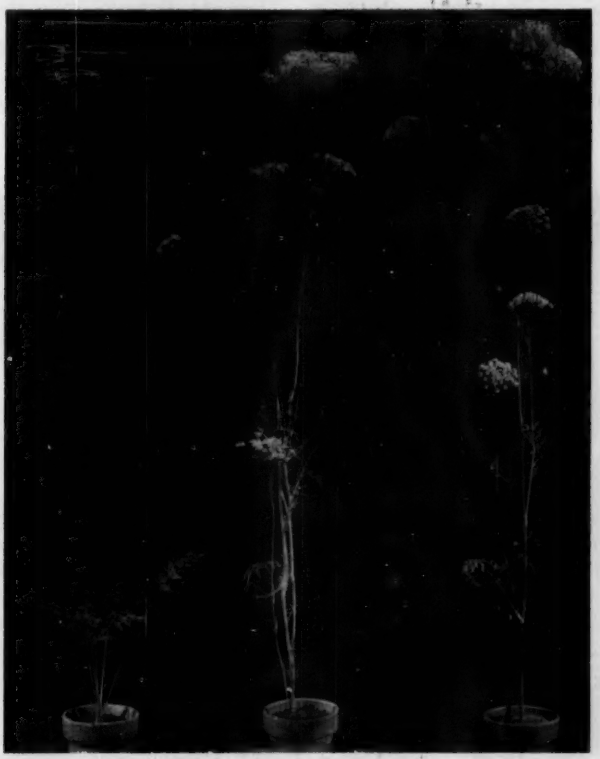

FIG. 1.—Carrot, Early French Forcing. *Left:* control; *right:* 8 weeks' cold treatment; *center:* 10 μg. of gibberellin daily. The flowering plants are 1 meter tall.

fertile. (Seed set in carrot was generally poor in the greenhouse.) In turnip, only flower buds had been produced at the end of 6 months of continuous treatment, while in parsley, only a few plants had formed microscopic inflorescences over the same period of time.

Petkus winter rye was treated for 3–4 weeks with gibberellin solutions ranging from 0.1 to 50 mg/liter. Stem elongation was markedly enhanced, particularly by the higher concentrations, the stem becoming thin and the leaves small. However, flower formation, as measured by the stage of ear development and by the number of leaves produced prior to ear initiation, was not significantly affected.

Results with Long-Day Plants.—The results with *Samolus*, *Crepis*, *Silene*, and annual *Hyoscyamus* are presented in Table 2.[2] *Samolus* and *Crepis* responded

TABLE 2

EFFECT OF GIBBERELLIN TREATMENT ON FLOWER FORMATION IN LONG-DAY PLANTS

Gibberellin (Micrograms/Plant/Day)		*Samolus parviflorus*	*Crepis tectorum*	*Silene armeria*	*Hyoscyamus niger* (Annual)
0	No. plants treated	32	9	12	25
	No. plants flowering	1*	0	0	0
	Weeks to anthesis	8	...	...	...
1	No. plants treated	29	8	3	4
	No. plants flowering	29	8	0	1†
	Weeks to anthesis	4 to 10	4 to 7	...	>26
2	No. plants treated	29	6	9	5
	No. plants flowering	29	6	0	1†
	Weeks to anthesis	4 to 7	5 to 8	...	>26
5	No. plants treated	12	6	5	...
	No. plants flowering	12	6	0	...
	Weeks to anthesis	4 to 5	4 to 5	...	...
10	No. plants treated	21	3	8	20
	No. plants flowering	21	3	1†	18†
	Weeks to anthesis	4 to 5	5 to 6	...	>26
20	No. plants treated	18	4	6	...
	No. plants flowering	18	4	3†	...
	Weeks to anthesis	4 to 5	4 to 6	9 to >20	...
50	No. plants treated	...	...	6	15
	No. plants flowering	...	...	6	15†
	Weeks to anthesis	...	...	12 to 15	>26
100	No. plants treated	6	...	6	...
	No. plants flowering	6	...	6	...
	Weeks to anthesis	4	...	14 to 18	...

* One plant flowered spontaneously for unknown reasons.
† Plants, or part of plants, failed to reach anthesis before end of experiment.

rapidly with flower formation, and the number of flowers or inflorescences and their development were similar to those in plants transferred to continuous long day (see Fig. 2). However, the seed set in *Samolus* was definitely below that of long-day-induced flowers. (The seed set of *Crepis*, like that of carrot, is usually poor under greenhouse conditions. However, gibberellin-induced inflorescences did form some viable achenes.) The optimum of the flower-inducing effect is reached at about 10 μg. of gibberellin daily. Higher doses result only in a slightly earlier response.

Silene took a longer period of time and larger doses of gibberellin to initiate flowers, the plants first making a considerable amount of stem growth (Fig. 3). However, those receiving 50 and 100 μg. of gibberellin daily all came ultimately to flowering. The inflorescences were much less compact than in long-day-induced plants, but the flowers were well developed and produced at least some viable seed. In annual *Hyoscyamus*, only part of the plants initiated flowers; the flower buds were abortive. Daily treatment with 10 μg. of gibberellin or more sometimes caused injury and death of the tip of the elongating stem, accounting in part for the incomplete response. However, it is evident that, as in the biennial plants, the

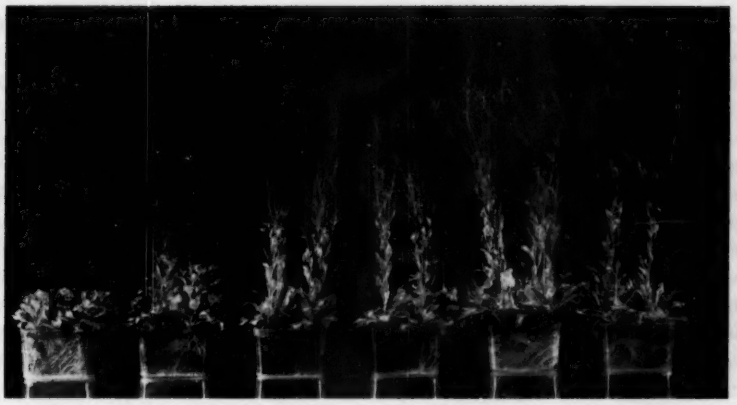

FIG. 2.—*Samolus parviflorus*. *Left to right:* controls; plants treated with 1, 2, 5, 10, and 20 μg. respectively, of gibberellin daily. The tallest plants are 40 cm. in height.

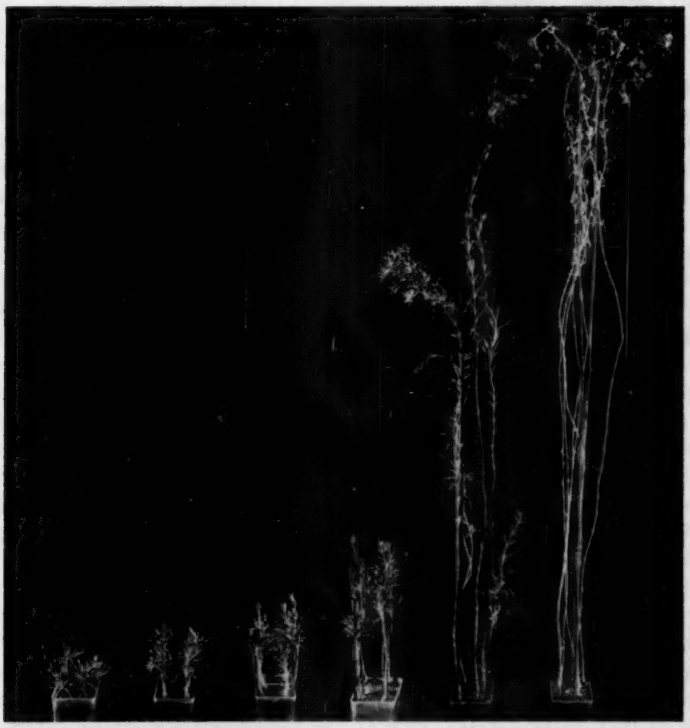

FIG. 3.—*Silene armeria*. *Left to right:* controls; plants treated with 2, 5, 10, 20, and 50 μg., respectively, of gibberellin daily. The tallest plant on the right is 1.5 meters high.

degree of flowering response which can be obtained by gibberellin treatment of long-day plants varies considerably, depending on the species.

Short-day-grown spring rye responded to gibberellin application similarly to non-cold-treated winter rye, that is, stem elongation was promoted but flower formation was neither promoted nor inhibited.

Experiments with Short-Day Plants.—Soybean and cocklebur were treated with doses of up to 10 μg. of gibberellin daily. The periods of treatment were 4 and 6 weeks, respectively. Stem elongation was very pronounced, the soybean plants assuming a twining habit of growth. However, the plants remained vegetative.

Specificity of the Gibberellin Effect.—In order to determine the specificity of the effect of gibberellin on flower formation in biennial and long-day plants, *Samolus*, *Silene*, and annual and biennial *Hyoscyamus* were treated with auxin (indoleacetic acid and naphthaleneacetic acid) and with kinetin, separately and in mixture. The maximal amounts (20 μg. of auxin, 2 μg. of kinetin) and the periods of application (4 weeks or more) were such that a similar treatment with gibberellin would have caused at least a pronounced bolting response. However, the plants continued to grow in the habit of rosettes and remained vegetative. The only visible effect was some epinasty of the petioles of the younger leaves in plants receiving relatively high auxin dosages.

DISCUSSION

Flower formation was induced in all four biennial plants and in four out of five long-day plants that were treated with gibberellin. No flowering response was obtained in the two short-day species that were tested, and flower formation was not affected in winter and spring rye. Positive results have also been observed in cabbage, rutabaga, and a number of other biennial plants;[3] in endive, a winter-annual plant;[4] and in *Crepis leontodoides*,[5] *Lapsana communis*,[6] dill, spinach, radish, and several other long-day plants.[3] On the other hand, no induction of flower formation was found in the short-day plant *Kalanchoë blossfeldiana*.[7] Of particular interest is the finding of Bünsow and Harder[8] in *Bryophyllum crenatum* and *B. daigremontianum*. These two species are long-short-day plants,[9] and gibberellin treatment enabled the plants to bypass the long-day part of photoinduction but apparently not its short-day part. It thus appears that application of gibberellin allows numerous plants to overcome cold and long-day requirements in flower formation but that it does not substitute for any short-day requirement.

These findings amplify our understanding of the role of gibberellin in growth and development of plants, and they provide us with a tool for the biochemical control of flower formation.

Gibberellin was discovered as a promoter of shoot elongation.[10] In the course of the last year, however, quite a variety of other gibberellin actions have been found: the effect in flower formation and effects on cell division,[11] seed germination,[12] fruit set,[13] inhibition and dormancy phenomena,[14] and others. On the other hand, the existence of gibberellin-like substances in flowering plants has been established by Phinney, West, Ritzel, and Neely[15] and others.[16] There seems little doubt that we have before us a new group of native plant regulators with a spectrum of functions which, quantitatively, is not less wide than that of auxin but is quite distinct from the latter qualitatively. The flower-inducing action of

gibberellin was not duplicated by treatment with auxin, kinetin, or mixtures of the two and seems therefore to be rather specific.

Induction or promotion of flower formation by treatment with defined chemical compounds has so far been accomplished only under highly special conditions or in isolated instances. Photoinduction in long-day plants can be promoted by treating the plants with sugars[17] or auxin,[18] but the effect is present only under photoperiodic threshold conditions and not in strictly noninductive daylengths. Auxin also promotes flower formation in the pineapple,[19] the litchi,[20] and certain varieties of sweet potato,[21] while maleic hydrazide was found to induce flower formation in young celery plants.[22] However, in numerous other plants the same compounds are either inactive with regard to flower formation or have an inhibitory effect on this process. The gibberellins are the first substances that have been found capable of inducing flower formation under unequivocally nonflowering conditions and in at least numerous representatives of large physiological groups of plants, the cold-requiring and the long-day plants. In view of the presence of gibberellin-like materials in higher plants,[15, 16] it seems reasonable to assume that such materials play an important part in the processes that lead to flower formation.

The precise function of the gibberellins in flower formation will have to be the subject matter of further study. However, several comments appear in order at the present moment. While the majority of cold-requiring and long-day plants that have been treated with gibberellin have responded with flower formation, some so far have not. This is true for rye, and Lona[5, 23] lists several other species (all long-day plants). In those species that did respond, the degree of response was variable, ranging from one similar to the effect of optimal thermo- or photoinduction to very late and weak flowering. This variation is not associated with differences at least in the photoperiodic sensitivity of the various species, for *Samolus*, *Crepis*, *Silene*, and annual *Hyoscyamus* do not differ appreciably in this respect. Two possible explanations come to mind. (*a*) The work of Phinney *et al.*[15] shows that the gibberellin-like materials from higher plants are not chemically identical with the known gibberellins, which are products of certain strains of the fungus *Fusarium moniliforme*. The evidence suggests the existence of a variety of different gibberellin-like substances in higher plants, and it is conceivable that these different gibberellins have some degree of species-specificity. In that case, a given plant may not respond optimally to a "foreign" gibberellin. (*b*) Gibberellin may not be the only factor limiting flower formation in cold-requiring and long-day plants but may be acting in conjunction with some other factor, for example, an inhibitory material. In such an event, the amount or activity of this second factor will determine the effectiveness of added gibberellin in flower formation. One preliminary result is in agreement with this assumption. In a small experiment with carrot and turnip it was found that the flowering response was more rapid and uniform when the temperature was relatively low, although not low enough to effect direct thermoinduction. A similar experience is reported by Wittwer and Bukovac.[3] It has been suggested that the effect of low temperature on flower formation in cold-requiring plants consists in changing the balance between promotive and inhibitory processes, low temperature shifting this balance in favor of the former.[24] If gibberellin is part of the "promotive processes," it should promote flower formation most actively at comparatively low temperatures. The reason for the failure

of some plants to form flowers upon gibberellin treatment may have been that these plants were treated under conditions in which the balance of the promotive and inhibitory processes of flower formation was too far on the side of inhibition.

Another question that will require careful study is whether the effect of gibberellin in flower formation is direct or secondary. Both thermo- and photoinduction result in the appearance of a transmissible floral stimulus in the plants. Attempts at isolating this material have failed, but results of grafting experiments suggest that the floral stimuli of biennial and of photoperiodically sensitive plants are different, while those of long- and short-day plants are interchangeable and thus, presumably, identical.[24] If this conclusion is valid, gibberellin, which causes flower formation in long- but not in short-day plants, is not the floral stimulus itself. It may, however, be a factor involved in the formation of this stimulus in long-day (and cold-requiring) plants. This interpretation is in agreement with a difference in the flower-inducing action of low temperature and long days, on the one hand, and of gibberellin, on the other. Most cold- and long-day-requiring plants, when grown under noninductive conditions, have a rosette habit of growth. All biennial and long-day species used in the present work belong to this type. Induction in these plants causes not only flower formation but also stem growth (bolting), and the two processes occur usually almost simultaneously. If the same plants are treated with gibberellin, stem elongation in most cases precedes flower initiation in a conspicuous manner. It appears from this that the primary effect of gibberellin is on stem growth and that flower formation is induced indirectly, the bolting plant becoming capable of forming floral stimulus.

SUMMARY

Treatment with gibberellin induced flower formation in four non-cold-treated biennial plants (biennial *Hyoscyamus niger*, carrot, turnip, parsley) and four long-day plants (*Samolus parviflorus*, *Crepis tectorum*, *Silene armeria*, annual *Hyoscyamus niger*) kept on short day. The degree of the flowering response varied from one similar to the effect of optimal thermo- or photoinduction to a late and incomplete response. Gibberellin treatment did not affect flower formation in unvernalized winter rye and short-day-grown spring rye and did not induce flower formation in two short-day plants (Biloxi soybean and cocklebur) maintained on long day. It is concluded that gibberellin-like materials play a part in the processes of flower formation but that they probably function in conjunction with other factors and that their effect on flower formation may be a secondary one.

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CHANGES IN PROTEIN TOPOGRAPHY UPON OXYGENATION

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The site of attachment of oxygen in the oxygen-carrying pigments has long been specifically identified as the metal in these proteins, the metal being attached to porphyrin groups in hemoglobin and chlorocruorin or directly to side chains of certain amino acid residues in hemerythrin and hemocyanin. It seemed of interest to inquire further whether the bulk of the macromolecule participates in the process of oxygen uptake. Recent work[1–6] has indicated that changes in optical rotation of proteins reflect modifications in over-all configuration. Measurements of specific rotation would thus seem in order.

A few measurements for hemoglobin are available in the older literature.[7, 8] The significance of the values observed [$\alpha^{20°}_{668\ m\mu} \simeq +15°$] is complicated by the presence of the porphyrin prosthetic group and of co-operative interactions between sites. Consequently it seemed appropriate to consider first the behavior of a pigment in which these complications do not occur. An examination was therefore made of the variations in optical rotation upon deoxygenation and oxygenation of hemocyanin, in which oxygen is held by copper attached directly to the protein fabric. In samples of this protein obtained from *Busycon*, the active sites act

independently of each other in isoionic solutions; the oxygenation curve fits a Hill equation[9, 10] with $n = 1$.

Since solutions of hemocyanin are deeply colored, measurements of optical rotation were made with a Bellingham and Stanley polarimeter to which a photomultiplier detector had been attached. Furthermore, in view of the strong absorption of yellow light by this protein, a mercury-cadmium lamp, rather than a sodium lamp, was used, with a strong line at 4358 A, near the minimum in the absorption curve of the protein.

Some observations are summarized in Table 1. Oxyhemocyanin from *Busycon canaliculatum* showed some small variations in specific rotation ($-56°$ to $-62°$) from sample to sample. A sample with $\alpha = -61.8°$ was treated with $NaHSO_3$, in an amount approximately five times the copper content of the protein solution. Within 15 minutes α dropped to $-34.5°$. Concurrently the blue color, and the associated absorption peak at 580 mμ, disappeared. Likewise, the much more intense absorption band of the oxygenated protein at 340 mμ vanished completely. When the solution was then allowed to stand open to air, the characteristic visible blue and the near-ultraviolet peak at 340 mμ of the oxygenated protein returned, though not to its full intensity, and α rose again to $-59.2°$.

Similar results were obtained with less $NaHSO_3$, in an amount about twice the copper content (Table 1).

TABLE 1

SPECIFIC ROTATIONS OF DEOXYGENATED HEMOCYANIN AND OXYHEMOCYANIN AT 25°C.

	Solution	Specific Rotation, α
1.	Oxyhemocyanin; dialyzed sera (approx. 1 per cent protein)	$-59.0°$, $-56.2°$, $-57.7°$, $-61.8°$
2.	Deoxygenated hemocyanin; Solution 1 + $NaHSO_3$; $NaHSO_3/Cu \simeq 5$	$-34.5°$
3.	Solution 2, exposed to air for 40 hours	$-59.2°$
4.	Oxyhemocyanin	$-62.3°$
5.	Deoxygenated hemocyanin; Solution 4 + $NaHSO_3$; $NaHSO_3/Cu \simeq 2$	$-37.7°$
6.	Oxyhemocyanin	$-59.5°$
7.	Deoxygenated hemocyanin; Solution 6 + glucose oxidase-catalase (0.003 per cent) and glucose (0.018 per cent)	$-21.8°$

As a further check, it seemed desirable to avoid a method of abstracting oxygen from the protein by reduction and to use a method of removal which depleted the dissolved oxygen in solution in equilibrium with the hemocyanin. Despite intensive efforts, complete displacement of dissolved oxygen by means of bubbled high-purity nitrogen could not be attained; although the weak 580 mμ peak of hemocyanin disappeared, it did not prove possible to reduce the intense 340 mμ absorption to the background value. An enzymatic method[11] was therefore used. A commercial preparation of glucose oxidase and catalase at a concentration of 0.003 per cent in the presence of 0.02 per cent glucose completely removed the oxygen from solution, as judged by the disappearance of the 340 mμ band of hemocyanin. As in the bisulfite treatments, this deoxygenated hemocyanin showed a pronounced drop in optical rotation, as compared to oxyhemocyanin (Table 1).

It is apparent, therefore, that in the oxygenation of hemocyanin, in which an O_2 molecule is bound at a specific localized site, the protein fabric is affected in a very extensive fashion. Such a possibility has also been suggested for hemoglobin, by Haurowitz[12] to account for crystallographic differences between ferro- and oxyhemoglobin, and by Wyman and Allen[13] and by St. George and Pauling[14] as an explanation of the co-operative interactions in the uptake of successive O_2 molecules. From the present experiments it is clear that substantial changes in topographical or structural configurations occur even in the absence of porphyrin prosthetic groups and even when the binding sites do not show any co-operative interactions. The very act of taking up an oxygen molecule makes itself felt over wide regions of the macromolecule.

The nature of these configurational changes is still obscure. The general trend has been to attribute them to steric displacements or rearrangements in polypeptide chains. It is now clear that these steric effects, if they exist, do not depend on the presence of a large porphyrin moiety. It is perhaps pertinent to point out also that the direction and magnitude of the change of optical rotation upon oxygenation are similar to those observed on denaturation of proteins and hence would imply a substantial increase in disorganization of the macromolecule. Nevertheless, oxygenation-deoxygenation processes can be carried through many cycles without appreciable change in the properties of the protein.

We should like to consider, therefore, an alternative model which attributes changes during oxygenation to modifications in the "frozen water"[15-19] of the hydration lattice of the oxygen-carrying protein. From this point of view, the binding site of the deoxygenated protein may be visualized as having an aqueous covering sheath whose lattice structure is determined by the nature of the residues (and metal) at the active site and by the amino acid side chains in the neighborhood of the site. Under appropriate conditions—for example, hemocyanin above its isoelectric point, or hemoglobin at any pH—these crystalline water islands may be sufficiently extensive that they merge into an interwoven lattice. The attachment of an oxygen molecule to the binding site would produce an incongruity in this lattice, because of the larger size[14] of O_2 as compared to H_2O and because of the differences in hydrogen-bonding properties. As a result, the hydration "iceberg" might be disturbed for an appreciable distance around the active site. This disturbance could affect the hydration structure of neighboring sites and hence change their affinity toward oxygen molecules. Simultaneously, it would manifest itself by changes in optical rotation. Changes in degree of interaction between sites of hemocyanin due to changes in pH may be attributed to changes in hydration as the charge of the protein is varied.

In terms of changes in the structure of the hydration lattice, one could also understand why so many different substances affect the affinity, and hence the shape and position of oxygenation curves, of the oxygen-carrying pigments. For hemoglobin these substances may vary from presumably innocuous salts[20] which affect only the ionic strength, to specific reagents such as mercurials[21] which combine with mercaptan groups, to generally reactive substances such as formaldehyde.[22] All these substances can *increase* the oxygen affinity of hemoglobin, and they affect the degree of interaction between sites; all these substances would also modify the lattice structure of the aqueous hydration sheath.

From this point of view the observed dielectric behavior of hemoglobin also seems reasonable. It has been reported by Takashima[23] that the high-frequency dielectric increment of hemoglobin changes with degree of oxygenation. As has been emphasized by Jacobson,[24] dielectric behavior reflects the character of the hydration sheath of a macromolecule. It is only reasonable, then, that binding of oxygen should change the dielectric properties if it affects the water lattice of the protein molecule.

It would seem, therefore, that the character of the "frozen" water on the surface of the protein may play an important role in determining the behavior of the oxygen-carrying pigments.

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*VECTOR MODEL FOR INDIRECT PROTON HYPERFINE INTERACTIONS IN π-ELECTRON RADICALS**

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Significant information on unpaired electron distributions in molecules, liquids, and solids can sometimes be obtained from magnetic resonance studies of electron-nucleus hyperfine interactions. A particularly important problem in this field involves the proton hyperfine interactions that have been observed in the electron magnetic resonance spectra of a large number of aromatic radicals in solution.[1-3] The problem concerns the theoretical basis for a proposal that the observed hyperfine splitting a_N due to aromatic proton N in an aromatic radical can be used to estimate the unpaired spin density ρ_N at carbon atom N to which proton N is attached, according to the equation[2, 4-6]

$$a_N = Q\,\rho_N. \tag{1}$$

A semiempirical value of Q derived from observed splittings a_N and theoretically calculated spin densities ρ_N is -30 ± 5 gauss, or -85 ± 15 Mc.[2] Equation (1) has been derived recently using molecular orbital theory with arbitrary π–π configuration interaction.[6] We show here how the simple proportionality in equation (1) may be derived using the Dirac vector model.

Let $\boldsymbol{S}_h$, $\boldsymbol{S}_\sigma$, $\boldsymbol{S}_\pi$ be the spin vector operators for electrons h, σ, π. Electron h is a 1s hydrogen atomic orbital centered on aromatic proton N. Electron σ is in the sp^2 hybrid orbital centered on carbon atom N and directed toward aromatic proton N. Electron π is in a $2p_z\pi$ orbital centered on carbon atom N. The nodal plane of the $2p_z\pi$ orbital passes through proton N. Let $|n^\circ>$ be a basic set of spin eigenfunctions including both σ and π electrons. Assume the Hamiltonian matrix H°_{mn} to be diagonal with respect to the set $|n^\circ>$ except for nondiagonal terms due to σ–π exchange coupling. Treat this σ–π interaction as a first-order perturbation on the ground-state spin function, $|0^\circ>$:

$$\mathcal{H}^{(1)} = \text{const.} - {}^1/_2(1 + 4\boldsymbol{S}_\sigma\cdot\boldsymbol{S}_\pi)J_{\sigma\pi}. \tag{2}$$

In equation (2) $J_{\sigma\pi}$ is the exchange integral between electrons σ and π. To first order, the exact ground-state spin function $|0>$ is

$$|0> = |0^\circ> + 2J_{\sigma\pi}\sum_n \frac{<n^\circ|\boldsymbol{S}_\sigma\cdot\boldsymbol{S}_\pi|0^\circ>}{\Delta E^\circ_n}\quad |n^\circ>, \tag{3}$$

where $\Delta E^\circ_n = E^\circ_n - E^\circ_0$. We use $|0>$ in equation (3) to calculate the hyperfine splitting due to proton N, which is

$$a_N = a^\circ_H\frac{<0|S_{hz}|0>}{S_z}. \tag{4}$$

Here, in standard notation,[5, 6]

$$a_H^\circ = \frac{8\pi g|\beta|}{3h}\frac{\mu_H}{I_H} \tag{5}$$

$$= 1{,}420 \text{ Mc}$$

and

$$S_z = \langle 0|\sum_k S_{kz}|0\rangle. \tag{6}$$

The sum in equation (6) is over all spins in the molecule. From equations (3) and (4) we obtain, to first order,

$$a_N = \frac{4J_{\sigma\pi}a_H^\circ}{S_z}\sum_n \frac{\langle 0^\circ|\boldsymbol{S}_\sigma\cdot\boldsymbol{S}_\pi|n^\circ\rangle\langle n^\circ|S_{hz}|0^\circ\rangle}{\Delta E_n^\circ}. \tag{7}$$

Explicit calculations show that only a very limited number of σ–π excited spin states can contribute to the summation in equation (7), and if we assume that all of these σ–π excited states have roughly the same excitation energy, ΔE, then equation (7) can be simplified to give

$$a_N = \frac{4J_{\sigma\pi}a_H^\circ}{\Delta E\, S_z}\langle 0^\circ|\boldsymbol{S}_\sigma\cdot\boldsymbol{S}_\pi S_{hz}|0^\circ\rangle. \tag{8}$$

To evaluate the above diagonal matrix element, we note that if $|\Pi^\circ\rangle$ is the normalized pure π-electron spin component of $|0^\circ\rangle$, then the σ component contains the factor $(\alpha(\boldsymbol{\sigma})\,\beta(\boldsymbol{h}) - \beta(\boldsymbol{\sigma})\,\alpha(\boldsymbol{h}))/\sqrt{2}$, and when the integrations over the spin variables of $\boldsymbol{h}$ and σ (and the other σ electrons) are carried out, we obtain

$$a_N = -\left(\frac{J_{\sigma\pi}}{\Delta E}\right)a_H^\circ\frac{\langle\Pi^\circ|S_{\pi z}|\Pi^\circ\rangle}{S_z}. \tag{9}$$

Since $\rho_N = \langle\Pi^\circ|S_{\pi z}|\Pi^\circ\rangle/S_z$,[6] we obtain equation (1) from equation (9) when $Q = -J_{\sigma\pi}\,a_H^\circ/\Delta E$. Previous calculations indicate $J_{\sigma\pi}/\Delta E$ to be of the order of magnitude of 0.1, so that this theoretical value of Q is in order-of-magnitude agreement with the semiempirical value.[5]

The critical step in the present derivation of equation (1) is the assumption of an approximately constant $\Delta E \approx \Delta E_n^\circ$ for all the interacting states $|n^\circ\rangle$ in equation (7). A sufficient condition for the validity of this assumption is that all π–π electron exchange integrals with magnitude $|J_{\pi\pi}|$ are very small compared with the CH bond integral $|J_{\sigma h}|$. In this case ΔE_n° is determined by $J_{\sigma\pi}$ alone. It is significant that the conditions we find here for the validity of equation (1) using the vector model are compatible with the molecular orbital derivation of equation (1), with arbitrary π–π configuration interaction.[6] In both cases the single proportionality between spin density and proton hyperfine splitting in equation (1) becomes exact as $|J_{\sigma\pi}|/|J_{\pi\pi}| \to 0$ and $|J_{\pi\pi}|/|J_{\sigma h}| \to 0$. Detailed calculations, including nonorthogonality effects, are of course required to test the adequacy of these limiting approximations.

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† Contribution No. 2214.

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CRITIQUE OF THE THEORY OF OPTICAL ACTIVITY OF HELICAL POLYMERS

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1. *Introduction.*—In a series of recent articles,[1–4] we have severally attempted to use coupled-oscillator theory in order to understand the optical activity of polypeptides. This approach, the development of which stems from Born[5] and Kuhn,[6] was first used in quantum mechanics by Kirkwood[7] as the basis for his polarizability theory of rotatory power. In recent months we have been clarifying the relationship between our respective treatments, leaving out of account differences which stem from the specific means by which we have attained tractability. As a result, we have been able to show that a general formula, derived by Kirkwood[7] under special (nondegenerate) conditions which do not apply to polymers, has a wider range of validity and may also be used in the present context. But, paradoxically enough, we have thereby found an error in Moffitt's work,[3] one of the purposes of which was to acknowledge explicitly the degenerate nature of this problem.

In the following section, Kirkwood's formula is rederived under conditions appertaining to a sequence of identical residues. And in a final section this prescription is more specifically applied to helices. Some discussion is given of the way in which the result may be used. It appears that a particularly important factor ($A_{\sigma\sigma}$) is composed of two terms, one of which ($A_{\sigma\sigma}^{(1)}$) was correctly assessed by Moffitt, whereas the other ($A_{\sigma\sigma}^{(2)}$) was absent from his earlier derivation. The reason for this omission is analyzed and shown to originate, somewhat unexpectedly, in the failure of the Born–von Kármán boundary conditions which he had used in the course of his exciton treatment.

We are forced to conclude that the close agreement between the data presented by Moffitt and Yang,[8] and Moffitt's calculations for the α-helix,[4] is illusory and does not enable a definite screw sense to be assigned to these polypeptides. Moffitt's calculations were based on certain $A_{\sigma\sigma}^{(1)}$ terms alone, and our estimates indicate that the corresponding $A_{\sigma\sigma}^{(2)}$ contributions are of the same order but of opposite sign. However, since the qualitative form of the dispersion shown by the various partial rotations was correctly described in his work, this quantitative failure in no way vitiates the empirical correlations put forward by Moffitt and Yang; any

reservations we may have on this score have already been enumerated by these authors.

2. *Degenerate Coupled-Oscillator Theory.*—In this section we shall rederive Kirkwood's formula in such a way as to include, explicitly, the case of identical residues. It is convenient to use a modified form of the notation employed by Moffitt;[3] thus we adopt Section 2 of that paper, with only a change of phases (see below), and those parts of Section 3 which precede the paragraph containing equation (24). We shall avoid specifying the particular geometry of the polymer at this stage, so that our results are equally valid for random coils and for helices.

Our polymer therefore consists of an array of N identical residues, each of which is identified by a serial index m. The ground and excited states of the mth residue are represented by the real functions ψ_{0m} and $\psi_{\sigma m}$, respectively.[9] It is supposed that the interaction between different residues is sufficiently weak—or that the symmetry of the array is sufficiently high—that the respective excitation energies $\epsilon_{\sigma m} = \epsilon_\sigma$ are the same for all residues.

The ground state of the polymer as a whole is represented, in zeroth-order, by the simple product $\prod_m \psi_{0m}(\tau_m)$, which we call $|0)$; τ_m symbolizes the co-ordinates of the electrons associated with the mth residue. At first, its excited states are also represented by simple products; if only one residue is excited ("fundamentals"),

$$|\sigma m) = |0)\,(\psi_{0m})^{-1}\psi_{\sigma m}; \tag{1}$$

if two or more residues are excited ("overtones" and "combinations"),

$$\begin{aligned} |\sigma m \tau n) &= |0)\,(\psi_{0m}\psi_{0n})^{-1}\,\psi_{\sigma m}\psi_{\tau n} \\ &\equiv |\tau n \sigma m), \qquad (n \neq m); \end{aligned} \tag{2}$$

and so on. Owing to the possibility of synchronizing the instantaneous charge distributions in different residues, these functions must be combined linearly to give the correct zeroth-order functions, namely,

$$\begin{aligned} |\sigma K) &= \sum_m |\sigma m)\,(\sigma m|\,\sigma K), \\ |\sigma \tau K) &= \sum_m \sum_{n \neq m} |\sigma m \tau n)\,(\sigma m \tau n|\,\sigma \tau U), \end{aligned} \tag{3}$$

where the concomitant transformations are all unitary and serve to diagonalize the Hamiltonian locally: typically,

$$(\sigma K|\,\mathcal{H}|\,\sigma K') = E_{\sigma K}\delta_{KK'}. \tag{4}$$

The stationary states of the polymer, correct to first-order terms, are now represented by

$$\begin{aligned} |0\rangle &= |0) + \sum_\rho \sum_\tau \sum_U |\rho \tau U)\,(\rho \tau U\|0), \\ |\sigma K\rangle &= |\sigma K) + \sum_{\tau \neq \sigma} |\tau L)\,(\tau L\|\sigma K) + \sum_\tau \sum_\rho \sum_W |\sigma \tau \rho W)\,(\sigma \tau \rho W\|\sigma K), \end{aligned} \tag{5}$$

with similar expressions for the upper states of combinations bands. Here

$$\begin{aligned} (\rho \tau U\|0) &= -(\rho \tau U|\,\mathcal{H}|\,0)/(\epsilon_\rho + \epsilon_\tau), \\ (\tau L\|\sigma K) &= -(\tau L|\,\mathcal{H}|\,\sigma K)/(\epsilon_\tau - \epsilon_\sigma), \\ (\sigma \tau \rho W\|\sigma K) &= -(\sigma \tau \rho W|\,\mathcal{H}|\,\sigma K)/(\epsilon_\rho + \epsilon_\tau), \end{aligned} \tag{6}$$

and it has been assumed that the numerators are first-order small quantities with respect to the denominators.

We now compute, correct to first order, the matrix elements of a typical "one-electron" operator $\mathcal{P}$, such as the linear or angular momentum, corresponding to the excitation of a fundamental. Setting

$$\begin{aligned} V_{mn}^{\sigma\tau} &= (\tau n|\,\mathcal{H}|\,\sigma m), \\ \mathcal{P}_{\sigma m} &= \langle 0|\,\mathcal{P}|\,\sigma m\rangle = (\sigma m|\,\mathcal{P}|\,0)^*, \end{aligned} \tag{7}$$

we find, after a little rearrangement, that

$$\begin{aligned} \langle 0|\,\mathcal{P}|\,\sigma K\rangle = \sum_m \{\mathcal{P}_{\sigma m} &- \sum_{n\neq m} V_{mn}^{\sigma\sigma}\mathcal{P}_{\sigma n}^*/2\epsilon_\sigma \\ &- \sum_{\tau\neq\sigma}\sum_{n\neq m} V_{mn}^{\sigma\tau}[\mathcal{P}_{\tau n}^*/(\epsilon_\tau + \epsilon_\sigma) + \mathcal{P}_{\tau n}/(\epsilon_\tau - \epsilon_\sigma)]\}\ (\sigma m|\,\sigma K). \end{aligned} \tag{8}$$

With our phase choice, we may enumerate two particular forms which this expression may take, depending on whether $\mathcal{P}_{\sigma m}$ is real or imaginary. If $\mathcal{P}$ is to represent the total linear momentum $\boldsymbol{P} = \sum_m \boldsymbol{p}_m$ (or, again, the total angular momentum $\boldsymbol{L}$), $\mathcal{P}_{\sigma m} = \boldsymbol{p}_{\sigma m}$ is imaginary and therefore

$$\begin{aligned} \langle 0|\,\boldsymbol{P}|\,\sigma K\rangle = \sum_m \{\boldsymbol{p}_{\sigma m} &+ \sum_{n\neq m} \boldsymbol{p}_{\sigma n} V_{mn}^{\sigma\sigma}/2\epsilon_\sigma \\ &- 2\sum_{\tau\neq\sigma}\sum_{n\neq m} V_{mn}^{\sigma\tau}\boldsymbol{p}_{\tau n}\epsilon_\sigma/(\epsilon_\tau^2 - \epsilon_\sigma^2)\}\ (\sigma m/\sigma K). \end{aligned} \tag{9}$$

On the other hand, taking $\mathcal{P} = \boldsymbol{Q} = \sum_m \boldsymbol{q}_m$, where $\boldsymbol{q}_m$ is the sum of the position vectors of all the electrons in residue m, we see that $\boldsymbol{q}_{\sigma m}$ is real, so that

$$\begin{aligned} \langle 0|\,\boldsymbol{Q}|\,\sigma K\rangle = \sum_m \{\boldsymbol{q}_{\sigma m} &- \sum_{n\neq m} \boldsymbol{q}_{\sigma n} V_{mn}^{\sigma\sigma}/2\epsilon_\sigma \\ &- 2\sum_{\tau\neq\sigma}\sum_{n\neq m} V_{mn}^{\sigma\tau}\boldsymbol{q}_{\tau n}\epsilon_\tau/(\epsilon_\tau^2 - \epsilon_\sigma^2)\}\ (\sigma m/\sigma K). \end{aligned} \tag{10}$$

Since ψ_{0m}, $\psi_{\sigma m}$ are the eigenfunctions of a self-consistent field operator $\mathcal{H}_m$,

$$\boldsymbol{p}_{\sigma m} = (m_e/i\hbar)\epsilon_\sigma \boldsymbol{q}_{\sigma m}, \tag{11}$$

where m_e is the electronic mass. Using this relation and the properties derived above, it is now easily verified that, correct to the first order,

$$\langle 0|\,\boldsymbol{P}|\,\sigma K\rangle = (m_e/i\hbar)E_{\sigma K}\,\langle 0|\,\boldsymbol{Q}|\,\sigma K\rangle, \tag{12}$$

where, for simplicity, we have taken as origin for the energy that of the ground state in zeroth-order. Thus the internal consistency of our model allows us to use with impunity either the dipole moment or the dipole velocity expressions relating to the optical properties of the polymer.

It is a straightforward matter at this stage to write down, correct to first-order, the rotational strength associated with any transition in the polymer. Since this quantity is the scalar product of two vector matrix elements, namely, of $\boldsymbol{Q}$ and of $\boldsymbol{L}$, it is clear that, to our approximation, at least one of these elements must be non-vanishing in zeroth-order. Accordingly, we may ignore the contributions from overtones and combinations, confining ourselves to the fundamentals whose rotational strengths are typified by

$$R_{\sigma K} = (e^2/2m_e c) Im[\langle 0|\, \boldsymbol{Q}\,|\, \sigma K\rangle \cdot \langle \sigma K|\, \boldsymbol{L}\,|\, 0\rangle]. \tag{13}$$

Since $\boldsymbol{L}$ is referred to a fixed origin, we write $\boldsymbol{L} = \sum_m \boldsymbol{k}_m$, preferring to reserve the symbol $\boldsymbol{l}_m$ for the angular momentum of the electrons of the mth residue, referred to an origin in that residue; we note that

$$\boldsymbol{k}_{\sigma m} \equiv (0|\, \boldsymbol{L}\,|\, \sigma m), \tag{14}$$

like $\boldsymbol{p}_{\sigma m}$, is a pure imaginary vector. Accordingly,

$$\begin{aligned} R_{\sigma K} = {} & (ie^2/2m_e c) \sum_m \sum_{m'} (\sigma m|\, \sigma K)\ (\sigma K|\, \sigma m') \\ & \times \{\boldsymbol{q}_{\sigma m} \cdot \boldsymbol{k}_{\sigma m'} + (2\epsilon_\sigma)^{-1} [\sum_{n \neq m'} \boldsymbol{q}_{\sigma m} \cdot \boldsymbol{k}_{\sigma n}\ V_{m'n}{}^{\sigma\sigma} - \sum_{n \neq m} \boldsymbol{q}_{\sigma n} \cdot \boldsymbol{k}_{\sigma m'}\ V_{mn}{}^{\sigma\sigma}] \\ & - 2 \sum_{\tau \neq \sigma} (\epsilon_\tau{}^2 - \epsilon_\sigma{}^2)^{-1} [\sum_{n \neq m'} \boldsymbol{q}_{\sigma m} \cdot \boldsymbol{k}_{\tau n}\ \epsilon_\sigma\ V_{m'n}{}^{\sigma\tau} + \sum_{n \neq m} \boldsymbol{q}_{\tau n} \cdot \boldsymbol{k}_{\sigma m'}\ \epsilon_\tau\ V_{mn}{}^{\sigma\tau}]\}. \end{aligned} \tag{15}$$

The partial rotation, per residue, due to this transition is therefore

$$[m_{\sigma K}] = \left(\frac{\hbar c F_\nu}{e^2 N}\right) \frac{\nu^2 R_{\sigma K}}{(\nu_{\sigma K}{}^2 - \nu^2)}, \tag{16}$$

where $\nu_{\sigma K} = E_{\sigma K}/hc$ is expressed in wave numbers and we have set

$$F_\nu = \frac{48 N_0 e^2}{\hbar^2 c^2} \left(\frac{n_\nu{}^2 + 2}{3}\right), \tag{17}$$

N_0 being Avogadro's number and n_ν being the refractive index of the medium at frequence ν.

If the frequency of the incident light is not too close to $\nu_\sigma = \epsilon_\sigma/hc$, so that, for all K, $|\nu_\sigma - \nu| \gg |\nu_{\sigma K} - \nu_\sigma|$, then the contribution to the residue rotation from transitions to all states K in the exciton band $\psi_0 \rightarrow \psi_\sigma$ is proportional to

$$\frac{\nu^2 \sum_K R_{\sigma K}}{(\nu_\sigma{}^2 - \nu^2)} - \frac{2\nu^2 \nu_\sigma{}^2}{(\nu_\sigma{}^2 - \nu^2)^2} \sum_K R_{\sigma K}(E_{\sigma K} - \epsilon_\sigma)/\epsilon_\sigma.$$

The summation in the first term reduces to

$$\begin{aligned} (ie^2/2m_e c) \sum_m \{ & \boldsymbol{q}_{\sigma K} \cdot \boldsymbol{k}_{\sigma m} - 2 \sum_{\tau \neq \sigma} \sum_{n \neq m} (\epsilon_\tau{}^2 - \epsilon_\sigma{}^2)^{-1}\ V_{mn}{}^{\sigma\tau} \\ & \times (\epsilon_\sigma \boldsymbol{q}_{\sigma m} \cdot \boldsymbol{k}_{\tau n} + \epsilon_\tau \boldsymbol{q}_{\tau m} \cdot \boldsymbol{k}_{\sigma m})\}, \end{aligned}$$

and, dropping second-order terms, the summation in the second becomes

$$(ie^2/2m_e c) e_\sigma{}^{-1} \sum_m \sum_{m'} \boldsymbol{q}_{\sigma m} \cdot \boldsymbol{k}_{\sigma m'} \sum_K (\sigma m|\, \sigma K)\ \langle \sigma K|\, \mathfrak{H} - \epsilon_\sigma|\, \sigma K\rangle\ (\sigma K|\, \sigma m').$$

Since $\mathfrak{H}$ is locally diagonal, the last line is just

$$\sum_K \sum_{K'} (\sigma m|\, \sigma K)\ \langle \sigma K|\, \mathfrak{H} - \epsilon_\sigma|\, \sigma K'\rangle\ (\sigma K'|\, \sigma m') = (\sigma m|\, \mathfrak{H} - \epsilon_\sigma|\, \sigma m') = V_{mm'}{}^{\sigma\sigma}$$

which vanishes if $m = m'$. Accordingly, the summation in the second term becomes

$$(ie^2/2m_e c) \sum_m \sum_{n \neq m} \epsilon_\sigma{}^{-1} V_{mn}{}^{\sigma\sigma} \boldsymbol{q}_{\sigma m} \cdot \boldsymbol{k}_{\sigma n}.$$

As a last transformation, we now put

$$\boldsymbol{k}_{\sigma m} = \boldsymbol{l}_{\sigma m} + \boldsymbol{r}_m \times \boldsymbol{p}_{\sigma m} = \boldsymbol{l}_{\sigma m} + (m_e\epsilon_\sigma/i\hbar)\boldsymbol{r}_m \times \boldsymbol{q}_{\sigma m}, \tag{18}$$

where $\boldsymbol{l}_{\sigma m}$ is the appropriate vector matrix element of the angular momentum referred to a point $\boldsymbol{r}_m$, fixed in the mth residue.

The partial rotation $[m_\sigma]$ may therefore be written as follows:

$$[m_\sigma] = [m_{\sigma i}] + [m_{\sigma j}] + [m_{\sigma h}] + [m_{\sigma h'}] + [m_{\sigma j'}], \tag{19}$$

where

$$\begin{aligned}
[m_{\sigma i}] &= \left(\frac{i\hbar F_\nu}{2m_eN}\right)\frac{\nu^2}{(\nu_\sigma^2 - \nu^2)}\sum_m \boldsymbol{q}_{\sigma m}\cdot\boldsymbol{l}_{\sigma m},\\
[m_{\sigma j}] &= -\left(\frac{F_\nu}{N}\right)\frac{\nu^2}{(\nu_\sigma^2 - \nu^2)}\sum_m\sum_{n\neq m}\sum_{\tau\neq\sigma}\frac{\epsilon_\sigma\epsilon_\tau}{(\epsilon_\tau^2 - \epsilon_\sigma^2)}V_{mn}{}^{\sigma\tau}(\boldsymbol{r}_m - \boldsymbol{r}_n)\cdot(\boldsymbol{q}_{\sigma m}\times\boldsymbol{q}_{\tau n}),\\
[m_{\sigma h}] &= -\frac{1}{2}\left(\frac{F_\nu}{N}\right)\frac{\nu^2\nu_\sigma{}^2}{(\nu_\sigma^2 - \nu^2)^2}\sum_m\sum_{n\neq m}V_{mn}{}^{\sigma\sigma}(\boldsymbol{r}_m - \boldsymbol{r}_n)\cdot(\boldsymbol{q}_{\sigma m}\times\boldsymbol{q}_{\sigma n}),\\
[m_{\sigma h'}] &= -\left(\frac{i\hbar F_\nu}{m_eN}\right)\frac{\nu^2\nu_\sigma{}^2}{(\nu_\sigma^2 - \nu^2)}\sum_m\sum_{n\neq m}\epsilon_\sigma{}^{-1}V_{mn}{}^{\sigma\sigma}\boldsymbol{q}_{\sigma m}\,\boldsymbol{l}_{\sigma n},\\
[m_{\sigma j'}] &= -\left(\frac{i\hbar F_\nu}{m_eN}\right)\frac{\nu^2}{(\nu_\sigma^2 - \nu^2)}\sum_m\sum_{n\neq m}\sum_{\tau\neq\sigma}(\epsilon_\tau{}^2 - \epsilon_\tau{}^2)^{-1}V_{mn}{}^{\sigma\tau}\\
&\qquad\times(\epsilon_\sigma\boldsymbol{q}_{\sigma m}\cdot\boldsymbol{l}_{\tau n} + \epsilon_\tau\boldsymbol{q}_{\tau n}\cdot\boldsymbol{l}_{\sigma m})
\end{aligned} \tag{20}$$

$[m_{\sigma i}]$ represents the intrinsic partial rotations of the individual residues, whose electrons are subject to slightly asymmetric Hartree fields, arising from any asymmetric centers they may possess or from their polymeric environment. If the transition $\psi_0 \rightarrow \psi_\sigma$ is associated with a large change in magnetic moment, and therefore a small dipole moment,[10] it is said to have a high dissymmetry factor;[11] for such bands, $[m_{\sigma i}]$ dominates the partial rotation, and the remaining terms in $[m_\sigma]$ may safely be dropped. $[m_{\sigma j}]$, which shows the same dispersive behavior as $[m_{\sigma i}]$, arises from the first-order corrections, $\{|\,\rangle - |\rangle\}$, due to the correlative interaction between states in different exciton bands. For strong electronic bands, this term can be large; in general, it is of the same order as $[m_{\sigma i}]$, though, in coupled-oscillator theory, one is inclined to hope that it outweighs $[m_{\sigma i}]$. The third contribution, namely, $[m_{\sigma h}]$, shows different dispersion and is a unique feature of the degenerate case; it arises from the correlative interaction between different residues, within the same exciton band. It was at this term that Moffitt attempted to direct attention.[3] The last two terms $[m_{\sigma h'}]$ and $[m_{\sigma j'}]$, are small with respect to their imprimed counterparts, namely, $[m_{\sigma h}]$ and $[m_{\sigma j}]$, by a factor $d_\sigma/|\boldsymbol{r}_m - \boldsymbol{r}_n|$, where d_σ is the distance of $\boldsymbol{r}_m$ from the partial optic axis of the transition $\psi_{0m} \rightarrow \psi_{\sigma m}$.[3] Following Kirkwood,[7] we therefore drop both of these.

It may be noticed that, on summing over all exciton bands, the residue rotation may now be written

$$\begin{aligned}
[m] &= \sum_\sigma [m_\sigma] = \sum_\sigma [m_{\sigma i}]\\
&\quad + \frac{1}{N}F_\nu\sum_m\sum_{n>m}\sum_\sigma\sum_\tau\frac{\nu^2\nu_\sigma\nu_\tau V_{mn}{}^{\sigma\tau}}{(\nu_\sigma^2 - \nu^2)(\nu_\tau^2 - \nu^2)}(\boldsymbol{r}_n - \boldsymbol{r}_m)\cdot(\boldsymbol{q}_{\sigma m}\times\boldsymbol{q}_{\tau n}),
\end{aligned} \tag{21}$$

where, by combining the $[m_{\sigma j}]$ and $[m_{\sigma h}]$ contributions, we have removed the restriction $\tau \neq \sigma$ from the summation over τ. Since $\nu_\sigma = \nu_{\sigma m}$, $\nu_\tau = \nu_{\tau n}$ for all m, n,

it will be recognized that this formula is identical with that obtained by Kirkwood.[7] But, whereas the validity of the original derivation was confined to the nondegenerate case, we see that this restriction has been removed by the present treatment. If $V_{mn}^{\sigma\tau}$ is replaced by an appropriate dipole-dipole approximation, the more particular results of the polarizability theory follow immediately.

3. *Application to Helices.*—Let us now specialize our discussion and suppose that the successive residues are arranged to form a right-handed helix. The monomers may then be located by the equivalent points $\boldsymbol{r}_m$ in each, namely, by

$$\boldsymbol{r}_m = \rho \cos \alpha_m \boldsymbol{i} + \rho \sin \alpha_m \boldsymbol{j} + mZ\boldsymbol{k}, \tag{22}$$

where P is the number of residues per turn (and not necessarily integral), PZ is the pitch of the helix, and $\alpha_m = 2\pi m/P$. Similarly, we may put $\boldsymbol{q}_{\sigma m} = |q_\sigma| \boldsymbol{e}_{\sigma m}$, where $\boldsymbol{e}_{\sigma m}$ is a unit vector whose radial, tangential, and vertical components, relative to the circumscribed cylinder, are $e_{\sigma r}$, $e_{\sigma t}$, and $e_{\sigma v}$, respectively:

$$\boldsymbol{e}_{\sigma m} = (e_{\sigma r} \cos \alpha_m - e_{\sigma t} \sin \alpha_m)\boldsymbol{i} + (e_{\sigma r} \sin \alpha_m + e_{\sigma t} \cos \alpha_m)\boldsymbol{j} + e_{\sigma v}\boldsymbol{k}. \tag{23}$$

We suppose the helix is sufficiently long that we may neglect end effects. To simplify the discussion, we confine our attention to two transitions, $\psi_0 \rightarrow \psi_1$ and $\psi_0 \rightarrow \psi_2$, say, supposing that contributions from these dominate the residue rotation. The optical rotatory dispersion is then described by

$$[m] = \left\{[m_{1i}] + \frac{\nu^2\nu_1\nu_2(A_{12} + A_{21})}{(\nu_1^2 - \nu^2)(\nu_2^2 - \nu_1^2)}\right\} + \frac{\nu^2\nu_1^2 A_{11}}{(\nu_1^2 - \nu^2)^2} + \left\{[m_{2i}] + \frac{\nu^2\nu_2\nu_1(A_{21} + A_{12})}{(\nu_2^2 - \nu^2)(\nu_1^2 - \nu_2^2)}\right\} + \frac{\nu^2\nu_2^2 A_{22}}{(\nu_2^2 - \nu^2)^2}, \tag{24}$$

or, equivalently, by

$$[m] = [m_{1i}] + [m_{2i}] + \left\{\frac{\nu^2\nu_1^2 A_{11}}{(\nu_1^2 - \nu^2)^2} + \frac{\nu^2\nu_1\nu_2(A_{12} + A_{21})}{(\nu_1^2 - \nu^2)(\nu_2^2 - \nu^2)} + \frac{\nu^2\nu_2^2 A_{22}}{(\nu_2^2 - \nu^2)^2}\right\}, \tag{25}$$

where

$$A_{\sigma\tau} = F_\nu \sum_{n(>m)} V_{mn}^{\sigma\tau}(\boldsymbol{r}_n - \boldsymbol{r}_m)\cdot(\boldsymbol{q}_{\sigma m} \times \boldsymbol{q}_{\tau n}). \tag{26}$$

In making a theoretical analysis of the "observed" dispersion curves for such an idealized system, one may adopt several different viewpoints which it is as well to enumerate.

It should be recognized, first of all, that the calculation of terms involving $A_{\sigma\tau}$ presents a very different problem from that of computing the $[m_{\sigma i}]$'s. The so-called one-electron theory[11] is primarily concerned with the latter task, whereas the coupled-oscillator theory is best suited to the former. For the present, we cannot attempt to assess the intrinsic rotatory parameters and are forced to regard these as outside the scope of our work. We are therefore faced with two alternatives.

On the one hand, we may hope that the $[m_{\sigma i}]$'s are appreciably smaller than the coupled-oscillator terms. In this case, we attribute the main part of the optical rotation to those terms of equation (25) which have been inclosed in braces. We

then base our calculations on the polarizability theory or make some other use of the optical properties of the individual residues—in particular, of their absorption spectra. This approach has been adopted by Fitts and Kirkwood in their work on polypeptides.[1, 2]

Alternatively, we may observe that $[m_{1i}]$ shows the same frequency dependence, namely, $\nu^2/(\nu_1^2 - \nu^2)$, as the term accompanying it in braces in equation (24); and similarly for $[m_{2i}]$. The other two terms, which involve A_{11} and A_{22}, show an unusual dispersive behavior, however. We may therefore hope, on the basis of the observed frequency dependence, to distinguish between these two different sets of dispersive terms. Adopting this approach, we attempt empirically to separate contributions to the rotation due to the terms in braces from those due to the remainder of equation (24). The latter are amenable to calculation, and we are enabled to avoid the $[m_{ei}]$ difficulty without having to neglect it. This avenue has been explored by Moffitt and Yang[3] in the present context.

It should perhaps be added that neither method is very satisfactory. Thus it may be questioned whether the existing data actually succeed in separating the abnormally dispersing terms from their normal counterparts. And, again, it is by no means clear that the $[m_{ei}]$'s make a negligible contribution to the observed rotation. However, while making these reservations, we continue to pursue such work, since it is unlikely that progress is to be made in any other way.

Returning to the helical problem, we evaluate $A_{\sigma\sigma}$ by using equations (22) and (23). It is found that

$$\begin{aligned} A_{\sigma\sigma} &= A_{\sigma\sigma}^{(1)} + A_{\sigma\sigma}^{(2)}, \\ A_{\sigma\sigma}^{(1)} &= -4F_\nu |q_\sigma|^2 \rho e_{\sigma t} e_{\sigma v} \sum_{n(>m)} V_{mn}^{\sigma\sigma} \sin^2 [\pi(n-m)/P], \\ A_{\sigma\sigma}^{(2)} &= F_\nu |q_\sigma|^2 Z(e_{\sigma r}^2 + e_{\sigma t}^2) \sum_{n(>m)} (n-m) V_{mn}^{\sigma\sigma} \sin [2\pi(n-m)/P]. \end{aligned} \quad (27)$$

This expression for $A_{\sigma\sigma}$ should be compared with that derived by Moffitt,[3] under the same assumptions. (It will be recalled that this term arises from correlative interactions within the same exciton band and is therefore directly related to the correct zeroth-order functions $|\sigma K)$.) He found only the term $A_{\sigma\sigma}^{(1)}$, so that his analysis is in error. Since his mistake is rather interesting, we shall conclude by showing how it arose.

Moffitt considered the helix as a one-dimensional crystal and employed exciton theory. By this method, the coefficients $(\sigma m/\sigma K)$ of equation (3), which determine the correct zeroth-order functions, are evaluated by adopting the Born–von Kármán cyclic condition, which leads to

$$(\sigma m|\sigma K) = N^{-1/2} \exp(2\pi i m K/N), \quad (28)$$

where K runs from 1 to N, the total number of residues. The procedure is standard in the treatment of the optical properties of molecular crystals, as well as in almost all of crystal physics. It is not, however, quite correct, when the crystal is not in fact re-entrant.

It is easy to see that the error incurred in the calculation of $(0|Q|\sigma K)$ by adopting this boundary condition is of order $N^{-1/2}$, although the main part of this term is of order $N^{1/2}$ and correctly given. In computing oscillator strengths per residue, which are proportional to $N^{-1}|(0|Q|\sigma K)|^2$, the error is therefore of order N^{-1},

which is indeed negligible. However, in the present context, we wish to compute rotational strengths per residue, which are proportional to $N^{-1}(0|\boldsymbol{Q}|\sigma K)\,|\,(\sigma K|\boldsymbol{L}|0)$. Now $(\sigma K|\boldsymbol{L}|0)$ involves the moments of the electronic displacements about some fixed (but arbitrary) point in the molecule; it therefore contains terms of order $N^{3/2}$ as well as of order $N^{1/2}$. (The former were innocuously symbolized by $N^{1/2}\alpha_\sigma$, $N^{1/2}\beta_\sigma$ in Moffitt's equations [48],[3] although, since the difficulty was not foreseen, they were not explicitly evaluated.) The scalar product of the $N^{3/2}$ terms of $(\sigma K|\boldsymbol{L}|0)$ taken with the $N^{1/2}$ terms of $(0|\boldsymbol{Q}|\sigma K)$ vanishes identically—as, clearly, it must, because the residue rotation is not proportional to the number of residues. The predominant $N^{1/2}$ terms of $(0|\boldsymbol{Q}|\sigma K)$ and the $N^{1/2}$ terms of $(\sigma K|\boldsymbol{L}|0)$ yield a contribution to the residue rotation of order unity, namely, the term involving $A_{\sigma\sigma}^{(1)}$. But the scalar product of the $N^{-1/2}$ errors of the former taken with the $N^{3/2}$ terms of the latter does not vanish and is therefore of order N; this again leads to a contribution of order unity to the residue rotation, namely, the neglected term $A_{\sigma\sigma}^{(2)}$. It may be noticed that the $N^{3/2}$ terms of $(\sigma K|\boldsymbol{L}|0)$ can involve only perpendicularly polarized components of $\boldsymbol{q}_\sigma$, whose cylindrical direction cosines are $e_{\sigma r}$ and $e_{\sigma t}$. These large moments are also perpendicularly directed, so that the neglected term cannot involve the vertical component $e_{\sigma v}$. Looking at equations (27), it is therefore clear why $A_{\sigma\sigma}^{(1)}$ was correctly assessed but $A_{\sigma\sigma}^{(2)}$ was missing.

We may conclude that whereas the application of the Born–von Kármán boundary conditions leads to a correct picture of the spectra of molecular crystals, they may not be used with impunity. In particular, they fail to give an accurate account of the optical rotatory properties of long helices.

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† Contribution No. 1421.

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[7] J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479, 1937.

[8] W. Moffitt and J. T. Yang, these PROCEEDINGS, **42**, 596, 1956.

[9] In the paper referred to in n. 3, the $\psi_{\sigma m}$ were defined to be pure imaginaries, so that the vectors used in discussing the properties of partial optic axes should all be real.

[10] For small groups the presence of a large magnetic moment virtually precludes a large electric moment and vice versa.

[11] Cf. E. U. Condon, *Rev. Mod. Phys.*, **9**, 432, 1937.

A REMARKABLE ACTION OF THE MUTANT "RUDIMENTARY" IN DROSOPHILA MELANOGASTER

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Since the early work of Morgan and his students[1] it has been known that the mutant *rudimentary* (*r*, 1, 54.5) is sterile in the female sex. Rudimentary females are therefore produced by outcrossing and backcrossing with *r* ♂ which are completely fertile. Morgan and Lynch proposed an adequate explanation: the homozygous *rr* locus, when acting during oögenesis, produces something in the egg cytoplasm which affects adversely the predetermination processes and prevents the eggs from developing. In the heterozygote $+/r$ the normal locus counteracts this "poisoning." The morphology of this lethal effect was recently studied by Counce.[2] She found in the eggs of some *r* alleles that development proceeded normally up to primary organ formation but stopped completely between 13–16 hours. Later it might start again, which explains the few rare offspring from *rr* ♀ already obtained by Morgan.

Fifteen years ago, while studying a new *r* mutant called r^{pzbl}, I obtained strange results. While most *rr* ♀ were sterile, some produced offspring. From the mating *rr* × *r* many normal daughters were obtained, a smaller number of normal sons, and a similar small number of *r* ♀ and *r* ♂. Rudimentary females mated to + ♂ produced many normal daughters and a few normal as well as a few rudimentary sons. Normal daughters of *rr* ♀ mated to *r* ♂ bred like heterozygotes $r/+$, and normal sons bred as + ♂. The first idea was, of course, "contamination." But many repetitions made with all precautions gave the same results, including the extreme sex ratio in favor of females. Many experiments were made to find enhancers or suppressors which might explain the facts, all without result, and the work was dropped for the time being. As a matter of fact, Morgan had already had a comparable experience. Breeding *r* ♀ with *r* ♂, he had one positive result, namely, an offspring consisting of one rudimentary daughter and one rudimentary son, but also two long-winged daughters. He assumed the latter to be due to the mother's not having been virgin. He mated the two unexpected females to rudimentary males and obtained both normal and rudimentary females and males, which he considered proof of prefertilization (1915, p. 241).[1] A few pages later (p. 245) he reports again on offspring of seventy-four rudimentary females. In one bottle a few long-winged females and males appeared, "which must be due to error or contamination." We shall see that the two results were not necessarily due to error or contamination but were typical for some rudimentary lines.

In *Drosophila* the cytology of the meiotic divisions in the egg is topographically similar to that in *Bombyx*, where we proved[3] the presence of a recessive locus which induces development involving a polar body. The two second maturation spindles are arranged in tandem, and in telophase four nuclei are found vertically to the surface (see Fig. 1, in which only the X chromosome is drawn). The innermost is the egg nucleus and the others the polar nuclei. The second and third nucleus come to lie near each other later and are called the "polar copulation nucleus," (PCN) which remains for a long time in the egg but is finally absorbed.

Now it is possible that in the r eggs of the heterozygous female $r/+$ fertilized by an r sperm, one of the effects of the presence of two r chromosomes is to cause the PCN to act as an autogamic zygote nucleus and, further, to move into the polar plasm of the developing egg together with the descendants of the proper rr fertilization nucleus. Thus the developing rr ♀ contains in its ovary rr cells, and also $r/+$ cells derived from the PCN of the F_1 egg. If all rr eggs of these rr ♀ were sterile, only those eggs could develop which contained the $r/+$ PCN nuclei, (Fig. 1).

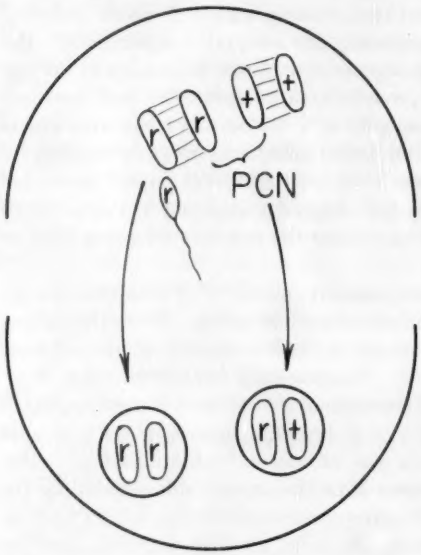

FIG. 1.—The two polar regions of a *Drosophila* egg. *Above:* egg nucleus and three polar bodies of an $r/+$ female; only the X chromosomes are drawn instead of nuclei. *Left:* fertilization of egg nucleus with r, by sperm with r. The two middle nuclei unite into polar copulation nucleus, PCN, which is $r/+$. At the opposite pole lies the polar plasma, determined to become the germ track. *Left arrow:* movement of descendant of normal fertilization nucleus into the germ track; *right arrow:* abnormal entrance of PCN nucleus into germ track.

If this nucleus behaved normally in the meiotic division, the offspring of such an $r/+$ egg from an rr mother would be females and males, one-half rudimentary, one-half normal. But there is also the possibility either that the PCN from F_1 remains together without meiosis or that, with correct meiosis, the new PCN takes over via autogamy (pseudoparthenogenesis), no fertilization occurring. All such eggs will develop into $r/+$ ♀. This sounds rather unusual. But recently Seiler[4] found in the moth *Solenobia triquetrella* that the parthenogenetic race develops from eggs with the PCN furnishing the embryonic nuclei. Thus it is to be expected that the offspring of exceptionally fertile r ♀ would consist of only $r/+$ daughters. But if most of the $r/+$ nuclei in the egg of an rr ♀ behave thus, but a few undergo meiosis and normal fertilization, a few rr ♀ and r ♂ and $+$ ♂ could be present.

The results of this set of experiments are tabulated in Table 1. Only 5 of the pair matings $rr \times r$, but 18 mass matings (20–30 rr ♀), were successful. Some such were made with $+$ ♂ (Nos. 1, 2, 4), others with r ♂ (No. 3). In No. 4 the rr mothers were not $F\,R_2$ ♀ but rr ♀ from more complicated crosses, which, however, must have had $r/+$ grandmothers—i.e., were identical in origin with the F_2 rr ♀. No. 3 shows the typical result of $rr \times r$ parents, the three others those of $rr \times +$ parents. Together there are 1,793 ♀, 52 ♂, a sex ratio of 34.5 ♀ : 1 ♂. We further see that exactly half the males are $+$ (r). In No. 3 there are also a few rr ♀, about the same number as of males in one of the male classes. This shows conclusively that over 1,700 are the products of pseudoparthenogenetic development of the grandparental PCN and that, in less than 3 per cent of the eggs, normal meiosis and fertilization had taken place. Only in No. 3 can the daughters from fertilization (rr) be seen; in Nos. 1, 2, and 4 they are also $r/+$.

In Nos. 5–8, tests for the + daughters of *rr* ♀ are given. Forty-two such daughters were mated to different kinds of males. All results show that the + daughters had been *r*/+. The numbers in the segregating classes point to normal meiosis and fertilization and not much pseudoparthenogenesis, if any.

TABLE 1

OFFSPRING OF FERTILE *r* ♀ FROM BACKCROSS (*N* × *r*) × *r* AND (FOURTH GROUP) *r* EXTRACTED FROM LATER GENERATIONS BRED FROM THE BACKCROSS; FURTHER TESTS (5–8) OF NORMAL DAUGHTERS FROM *rr* MOTHERS

	PARENTS	PAIR MATINGS	MASS MATINGS	FEMALES +	FEMALES *r*	MALES +	MALES *r*
1.	*r* ♀ × ♂$^+$	5	...	27	...	1	...
2.	*r* ♀ × ♂$^+$	...	7	910	...	8	10
3.	*r* ♀ × ♂ *r*	...	6	349	9	7	7
4.	*r* ♀ (later gen.) × ♂$^+$	...	5	506	...	10	9
5.	♀ + from *rr* × *r*, ♂ *r*	17	...	792	495	707	469
6.	♀ + from *rr* × +, ♂ brother *r*	4	...	262	143	191	122
7.	♀ + from *rr* × *r* stock ♂	16	...	831	286	597	522
8.	♀ + from *rr* × brothers +	5	...	365	...	207	69

At the time these experiments were made, only one other *r* stock, r^6, was tested on a small scale, with the same results. Neither of these stocks still exists. But it appeared advisable to repeat the experiments with dominant markers in the X chromosomes of the non-*r* grandparents, which should reappear in F_3 because they are derived from the grandparental (F_1) PCN, though not visibly present in the *rr* F_2 ♀. Unfortunately, it turned out that one line, r^9, had a considerable amount of fertility of *rr* ♀, while the other, r^{39}, produced almost completely sterile *rr* ♀ without any development of a PCN. But in a very large set of experiments r^9 gave a single case of PCN development which did not admit of any other explanation. (The almost complete sterility and the presence of the *rr* parents exclude any contaminations, apart from the types themselves.) The figures for this test are as follows: 127 sterile bottles with ±20 *rr* ♀; 198 fertile bottles with ±20 *rr* ♀: 3,111 *rr* ♀, 1,641 *r* ♂ in all; sex ratio approximately 2 ♀ : 1 ♂; average flies in bottles: (*a*) from r^9/r^9 ♀, 21; (*b*) from r^9/r^{39} ♀, 8; (*c*) from r^{39}/r^{39} ♀, 0.2. The only case of PCN development was 6 ♀ *B* +/+ *r*, 1 ♂ *B* +.

Although this is thus far the only successful experiment using a dominant marker in the X chromosome, the experiments with r^9 and r^{39} yielded an additional number of instances of PCN development, with the result that rudimentary females produced nonrudimentary offspring. The rarity of the phenomenon for r^9 and r^{39} crosses, as opposed to r^{nxbl}, becomes visible in the total result of half-a-dozen instances while testing almost 8,000 *rr* ♀. Actually, the following cases could not have been explained if the foregoing facts had not been known; thus they afford further proof for the analysis. Backcrosses (+ Oregon × *B f* r^{39}) × r^9 were made, and the rudimentary females (= *B f* r^{39}/r^9; also heterozygous for *B f*) were mated in mass (*ca.* 20 ♀) to r^9 ♂. Of 6 fertile bottles, 5 gave the expected result, i.e., rudimentary females and males, the females half with the *B*/+ phenotype, the males half *B f r*. There was an unusual extreme preponderance of females, which remains unexplained. One bottle contained completely aberrant offspring, namely (phenotypes): 95 ♀ *rr*, 113 ♀ *B*/+, 3 ♀ + +, 1 ♀ *B*/+ *rr*, 1 ♀ *B*/*B*, 88 ♂ *B f*, 88 ♂ *r*, 14 ♂ *B f r*, 2 ♂ +. It is obvious, first, that *rr* ♀ mated with *r* ♂ produced largely nonrudimentary offspring, second, that the big noncrossover

classes prove that the *rr* mothers had produced gametes half *B f*, half *r;* and, third, that unusually large numbers of offspring had come from a fertile *rr* ♀. The non-rudimentary classes show, in view of the former discussion, that they must have originated from *B f/r* nuclei in the gonad of an *rr* ♀, which could get there only by activation of the PCN nucleus of the grandmother. This nucleus should have been $+/B\,f\,r$; but, by crossing over in the F_1 ♀, it could have been in an individual egg *B f* or *r*. Fertilized by *r* in the backcross, a rare R F_2 ♀ could be *B f/r*—i.e., not rudimentary—and therefore could not have been the mother of the F_3. If, however, in the F_1 ♀ the crossover PCN had been activated, as described, an R F_2 rudimentary female would have nuclei in the ovary of the constitution *B f/r*, and this is borne out by the experiment. The crossover classes + and *B*/+ *rr* (phenotypes) would be the result of ordinary crossing over in the *B f/r* germ-track nuclei of the R F_2 ♀, and also the male classes *B f r* and + with the surprisingly large *B f r* class. The one *Bar* female might be a purely phenotypical variant of *B*/+ or a double crossover.

Exactly the same thing happened once more in another combination, namely, *rr* ♀ from the backcross (+ (Canton) × *B f r*)r^9 mated to *B f r* ♂. Eleven times (against 22 sterile bottles) the expected results were obtained: females *B f r*/*B f r* and *B f r*/*r*, males *r* and *B f r* and a few crossovers, all rather small numbers (all together, 64-80-26-25, 13 crossovers), as expected in the presence of r^{39}. One cross was aberrant and clearly derived from two fertile mothers, one with small but normal offspring, the other with larger offspring from a *B f/r* mother, the origin of which must have been the same as in the last case. The data are as follows: No. 4645: females *B f r*/*B f r* 39, *B f r*/*r* 27, *B f r*/*B f* 61, *B f r*/+ 12, *B f r*/*B* 1; males *r* 59, *B f r* 5, *B f* 68. A mother *B f* +/+ + *r* mated to *B f r* ♂ would produce the second and third classes of females and the first and third classes of males. A normal mother would produce the first group of females and part of the second group, and part of the first group of males and the second group. But this latter may also be a crossover class from the first females with PCN nuclei, as are the females of the fourth group. Again one double crossover female is found.

By chance, an analogue of the last case also was found. In the last example, a crossover egg from *B f r*/+ → *B f* +/+ + *r* was the beginning of the unexpected features. In the present case the backcross, from which *rr* ♀ were selected for mating with *r* ♂, was (*B*/*B* × r^9) × r^9, which, in the absence of r^{39}, produces many fertile *rr* daughters. Out of 18 mass matings with ±20 *rr* ♀, 17 gave the expected result: 404 *rr* ♀, 127 *r* ♂. But one bottle segregated into 65 *rr* ♀, 31 *r* ♂, 83 + ♀, 39 + ♂. The first idea is contamination; but this is excluded, since the only error imaginable, contamination with a *B*/*r* sister, could not produce + offspring. The relatively high fertility and the sex ratio indicate that we have the same situation as in the last case: the F_2 mother came from a crossover egg (*B* +/+ *r* → *B r*/+ +) with the + chromosome. Again the PCN *r*/+ was activated, and the F_2 rudimentary mother had *r*/+ nuclei in the germ track.

Finally, a few more complicated cases were found, proving that other variations exist in regard to the abnormal behavior of the PCN. The following case is remarkable, though the numbers are very small, due to the presence of r^{39}. The mating is *B*/+ *rr* ♀ (phenotype) from the backcross (Canton × r^9) × *B f* r^{39} with r^9 ♂. Expected are females *rr* and *B*/+ *rr*, males *r* and *B f r*. Only 8 flies

of the expected types hatched; in addition, one female of the phenotype $B/+$ and one ♀ $+$. Both of these unexpected females were successfully mated to r^9 ♂. The $B/+$ ♀ produced daughters (phenotypes) half $B/+$ rr, half $+$ and a few rr ♀, sons half $+$, half $B\ f\ r$. This female, then, had been $B\ f\ r/+$. As the mother should have been $B\ f\ r/r$, a $+$ chromosome can be obtained only if there was unequal crossing over of r, which is improbable as far as we know, or if the $+$ polar body of F_1 was fertilized by a $B\ f\ r^{39}$ sperm and then moved into the germ track as did the PCN in the other cases. The other unexpected female, the $+$ ♀, was mated to r^9 and produced females and males half $+$, half r. This female had then been $r/+$, which is possible if it was derived from the F_1 PCN in the typical way. The interpretation seems unavoidable and shows that in the presence of r^{39} three different kinds of nuclei went into the germ track of the F_1 ♀: normal ones, a PCN nucleus, and a polar-body nucleus fertiiized by sperm. In the same series a second identical case occurred; from $B\ f\ r/r$ ♀ mated to r^9 ♂, 4 expected flies originated and 2 females $+$ and 1 female BB or $B/+$ (intermediate in phenotype). The unexpected females were mated to r ♂ and again turned out to be of the constitution $+/r$ and $+/B\ f\ r$, respectively. The occurrence of 2 such unexpected cytological abnormalities in 7 fertile $rr \times r$ matings (out of 17) is remarkable.

Discussion.—The phenomenon here described consists in the activation of the normally perishing polar copulation nucleus (second and third polar bodies) under the influence of the mutant *rudimentary*, and its entering the germ track of the F_1 ♀, thus making it possible that a homozygous rudimentary female produces r and $+$ gametes. In the mutant r^{pzbl}, females rr become fertile only if this process saves them from sterility. In the mutants r^9 and r^{39} the same phenomenon can occur but only rarely, while fertile rr ♀ behave normally as a rule, though only a few offspring survive. Actually about 8,000 rr ♀, namely, r^9r^9, r^9r^{39}, and $r^{39}r^{39}$, were tested in batches of about 20 females (all single-pair matings made resulted in a single son). More than half of these were sterile, and it can be estimated that not more than 400 individual females produced offspring at all. Among these, only 5 cases of PCN development occurred, i.e., 1–2 per cent of reproducing females and only a few hundredths of 1 per cent in all females tested. As a whole, the phenomenon belongs to the group in which recessive mutants also affect the cytological behavior of the meiotic divisions in the egg, of which the best-analyzed case, both genetically and cytologically, is the mosaicism in the silkworm (Goldschmidt and Katsuki[3]). But, while here the action requires the homozygous recessive mutant (for oily skin), in the present case the mutant, r, acts in the heterozygote. It is the specific features of the insect development, namely, germ-track determination by polar cytoplasm into which cleavage nuclei immigrate and further the formation of a PCN, which make possible the surprising details of the abnormal behavior. It is remarkable that different r alleles act differently, and it can hardly be doubted that other, not yet tested alleles may present other variants of the phenomenon.

Summary.—The mostly sterile females homozygous for the rudimentary alleles r^{pzbl} and r^6, if fertile, produce large numbers of wild-type daughters, a few such sons, and a few rudimentary daughters and sons. The $+$ daughters are genetically $+/r$. The explanation is that only those eggs of rr ♀ develop that are derived from $r/+$ nuclei in the rr ovary. These originate from the polar copulation nu-

cleus of the grandmother $r/+$ which exceptionally entered the germ track. If these $r/+$ eggs develop by pseudoparthenogenesis (autogamy)—a possibility known from *Solenobia*—the offspring is female and wild type. If occasionally a few such nuclei undergo normal meiosis and fertilization, the possible classes of daughters as well as sons appear in small and equal numbers. A repetition with the alleles r^9 and r^{39} gave only a few such cases in about 8,000 females tested; r^{39} females are almost sterile, r^9 females relatively fertile. All together, about 1 female in 20 was fertile. In many of these, the grandmaternal X chromosome was marked with *Bar*, but only once were *Bar* offspring obtained from non-*Bar rr* ♀ by the activation of the grandmaternal polar copulation nucleus. A few more such cases occurred with complications by previous crossing over, all together in less than 2 per cent of the fertile females.

[1] C. J. Lynch, *Genetics*, **4**, 501, 1919; T. H. Morgan, *Z. indukt. Vererbungs.*, **7**, 323, 1912; *Am. Naturalist*, **49**, 240, 1915.

[2] S. J. Counce, *Z. indukt. Vererbungs.*, **87**, 443, 1956 (3 parts).

[3] R. Goldschmidt and K. Katsuki, *Biol. Zentr.*, **47**, 45, 1927; **48**, 39, 685, 1928; **51**, 78, 1931.

[4] J. Seiler, *Arch. Julius Klaus-Stift. Vererbungsforsch.*, **28**, 40, 1953.

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*SPECIAL PROBLEMS INHERENT IN THE STUDY OF HUMAN GENETICS WITH PARTICULAR REFERENCE TO THE EVALUATION OF RADIATION RISKS**

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Studies on man have contributed relatively little to the flowering of genetics which has characterized twentieth-century biological science. The reasons for this are well known: the length of the generation time in man, the impracticability of test matings, the difficulties in controlling environmental variables, the role of cultural overlay in molding and obscuring genetic predispositions. There are, in my opinion, numerous evidences today that despite these drawbacks, the study of human genetics has finally come of age, to the point where studies on man will yield results on a level of significance with those emerging from studies of other organisms well known to the geneticist. These results will emerge, not from looking to man for the qualities which have made *Drosophila* and *Neurospora* so useful to the geneticist, but, in part at least, from taking advantage of certain special attributes of man and the populations in which he gathers, attributes not shared by other organisms.

In considering the factors which have contributed to the coming of age of human genetics, it is convenient to distinguish between developments which are related to the "natural" evolution of the subject and other developments which are currently contributing to a somewhat "forced" evolution. As regards the "natural" evolution of human genetics, three contributory factors are outstanding. The first is

the recent rapid expansion of medical and biological knowledge, with the result that in the evaluation of a problem involving the genetics of man there can be brought to bear a set of techniques which far exceed those available for any other organism. The second is the development, during the last twenty years, of statistical techniques which enable a maximum of genetic information to be extracted from two-generation data, such as one must often be content with in human genetics. The third factor of importance in the recent "natural" evolution of human genetics is the changing content of medical practice, with the increasing control of contagious and parasitic diseases and the growing relative importance of congenital, constitutional, and degenerative diseases, to all of which genetic factors are so important.

As regards the "forced" evolution of human genetics, it is apparent that the very lively discussions of the past several years concerning the genetic problems created by increasing exposure to ionizing radiation have gone far to focus attention on the absolute necessity of acquiring as soon as possible a far better understanding of human genetics than we now possess. These same discussions have also stimulated many able geneticists to direct a greater portion of their creative energies toward human genetics than would otherwise have been the case, and to give serious consideration to the special problems of this field, a development from which the field cannot but benefit. The activities of the National Academy of Sciences Committee on Radiation Genetics are a notable example of this.

My assignment today is to review the special problems inherent in the study of human genetics. An exhaustive treatment of this subject would obviously be extremely time-consuming. Under the circumstances, I have chosen to restrict my remarks to the special problems which arise in connection with the subject of today's symposium, the evaluation of the genetic risks of increasing exposure to ionizing radiation.

In the evaluation of this problem there are certain types of information which are indispensable, in the sense of supplying the figures fed into mathematical treatments of the subject. These are (1) the spontaneous rate of mutation in man, be it on a per locus or a per gamete basis; (2) the induced mutation rate per locus or per gamete per unit of ionizing radiation; (3) the "accumulation factor," i.e., the ratio of deleterious mutations already present in the population to those arising spontaneously each generation through mutation; and (4) the manner of action of natural selection on past and contemporary human populations. Let us now consider briefly some of the special problems inherent in furthering our information concerning man in each of these four areas.

The Spontaneous Rate of Mutation of Human Genes. The spectrum of phenotypic effects resulting from gene mutation ranges from complete lethality to extremely minute effects, apparent only under special circumstances. Two of the standard techniques used in the study of mutation rates in sexually reproducing forms consist (1) in the use of a special strain of animals in which one or more chromosomes carry complex chromosomal rearrangements associated with so-called "marker" genes, of particular use in the detection of mutations associated with lethal effects, and (2) in the use of special strains homozygous for a number of recessive mutations involving known loci, of particular use in the detection of mutations at these specific loci. Neither of these techniques is available for man. One is therefore forced to

rely on the very simple device of counting the rate of appearance of a variety of mutant phenotypes in human populations and then computing the mutation rate, either on the basis of the gene replacement rate necessary to offset the reproductive disadvantage accruing to the phenotype or, in the case of dominantly inherited traits, on the basis of the proportion of sporadic cases. The former approach requires the assumption that the population is in genetic equilibrium or nearly so. In the case of phenotypes determined by recessive genes, the former approach also necessitates certain critical assumptions concerning the effect of the gene when heterozygous.

In order to avoid making such assumptions, which may introduce indeterminate errors, the tendency in recent studies on human mutation rates has been to concentrate on phenotypes determined by dominant genes and to base the estimate solely on the proportion of sporadic cases of the disease occurring in a given population over some arbitrary time interval. Now the situation is simpler, but still, before one can argue from the frequency of the trait in the population to the mutation rate, one must meet two important qualifications.

First, one must establish how frequently the phenotype in question is not the result of a germinal mutation. This may be difficult, but to fail to do so may introduce an error. A single specific example will suffice. Retinoblastoma is a type of cancer of the eye, developing at an early age, which in many family studies has been shown to behave as if due to a single dominant gene with a high degree of penetrance. Some years ago our group carried out a state-wide survey in Michigan, with reference to the frequency of occurrence of "sporadic" cases—i.e., individuals with no family history.[1] We then calculated a mutation rate on the assumption that each of these individuals represented a mutational event. We specified at the time that this estimate would be incorrect if some of these individuals owed their disease to nongenetic causes or to somatic mutations, but we had no evidence that this was the case. Subsequently, several studies have suggested that when the offspring of affected individuals who have reproduced are studied, there are fewer affected children than would be expected if all affected individuals owed their disease to germinal mutation.[2, 3] Our own estimate was consequently too high. This potential source of error is not serious, in the sense that the means of dealing with it are both clear and feasible, although temporarily, as in our case, the necessary data may not be at hand.

Second, for an estimate to be entirely valid, one must establish the fact that the mutant phenotype in question always results from mutation at the same locus. This is not possible in man at the present time, so that all studies to date may actually be measuring the sum of mutation at several loci. However, developments are in sight which will in some measure meet this problem. These are of two types. Studies on the linkage relationships of the gene(s) responsible for a given phenotype will reveal how many different loci may give rise to mutations resulting in that phenotype. For instance, elliptocytosis is a condition in which the erythrocytes are oval rather than round. Many extensive pedigrees are known in which the trait behaves as if due to a dominant gene. It has now been shown that in some of the pedigrees this gene behaves as if closely linked with the locus or loci responsible for the Rh serological reactions, while in other pedigrees there is no apparent linkage (see references in Morton[4]). The logical conclusion is that there are at least two genetic loci at which mutation may give rise to the ovalocytic trait. The second approach to a determination of the number of different loci at which there may

occur genes resulting in a given phenotype involves careful clinical studies of the trait. For instance, it has now been shown that there are really two types of hemophilia, both sex-linked in their inheritance, with the serum from an individual of one type able to correct the bleeding defect in the other type (see references in Graham,[5] Macfarlane,[6] Schulman and Currimbhoy[7]). One type is about four times as frequent as the other. Studies were carried out some ten to twenty years ago on the rate of mutation to the hemophilia gene.[8, 9] If the mutations resulting in these two types of hemophilia are ultimately shown to involve different loci, then the mutation-rate estimates resulting from these early studies must now be revised downward, to take into account the fact that two different loci are involved (cf. Graham[5]). As another example, diabetes insipidus, a condition characterized by the excretion of large quantities of very dilute urine, frequently behaves as if due to a sex-linked recessive gene. It has now been shown that in some pedigrees typical of sex-linked inheritance, the condition can be remedied by the administration of pitressin, an extract of the pituitary gland. In these pedigrees the primary defect would seem to be in the pituitary gland. In other pedigrees the condition does not respond to pitressin. Here the primary defect apparently resides in the renal tubule.[10] Clinical tools again allow us to differentiate two distinct types of the disease, a fact to be reckoned with if mutation-rate studies on this condition were contemplated.

Thus far, studies have been carried out on the rate of appearance through mutation of about a dozen different dominantly inherited phenotypes. The results are summarized in Table 1. It is an interesting fact that despite all the qualifications

TABLE I

ESTIMATES OF THE RATE OF APPEARANCE THROUGH MUTATION OF CERTAIN GENES IN MAN*

CHARACTER	POPULATION	MUTANT GENES PER 100,000 GAMETES	REFERENCE
		Autosomal Dominants	
Aniridia	Denmark	0.5	Møllenbach, 1947 Penrose, 1957
Chondrodystrophy	Denmark	4.3–4.9	Mørch, 1941
	Sweden	7	Böök, 1952
	Japan	9–14	Neel and Schull, unpublished
Dystrophia myotonica	Northern Ireland	0.8	Lynas, in press
Epiloia	England	0.8–1.2	Penrose, 1936
Marfan's syndrome	Northern Ireland	0.5	Lynas, unpublished
Microphthalmos without mental defect	Sweden	0.5	Sjögren and Larsson, 1949
Multiple polyposis of the colon	Michigan	1–3	Reed and Neel, 1955
Neurofibromatosis	Michigan	10	Crowe, Schull, and Neel, 1955
Pelger's nuclear anomaly	Germany	2.7	Nachtsheim, 1953
Retinoblastoma	England	1.4	Philip and Sorsby, 1944
	Michigan	2.3	Neel and Falls, 1951
	Germany	0.4	Vogel, 1954
Waardenburg's syndrome	Holland	0.4	Waardenburg, 1951
		Sex-Linked Recessives	
Hemophilia	England	2	Haldane, 1935, 1948
	Denmark	3.2	Andreassen, 1943
Pseudohypertrophic muscular dystrophy	Utah	10	Stephens and Tyler, 1951
	Northern Ireland	4.5–6.5	Stevenson, 1953
	Germany	4	Becker and Lenz, 1955
	England	4.3	Walton, 1955

* After J. V. Neel and W. J. Schull, *Human Heredity* (Chicago: University of Chicago Press, 1954); L. S. Penrose, in *Proceedings of the First International Congress of Human Genetics* (*Acta Genet. et Statist. Med.* [Suppl.], **6**, 169–182, 1956); A. C. Stevenson, *World Health Organization Publ.* (in press).

I have erected, the average of these estimates, about 3×10^{-5}, is not very different from the average commonly quoted for *Drosophila*, about 2×10^{-5}. However, because of these very qualifications, as well as certain other methodological difficulties involving *Drosophila* as well as man, it would perhaps be wise to regard this as an interesting coincidence rather than as a demonstration of similarity, until such time as much more extensive data are available.

In certain considerations it is important to have an estimate of the "total" mutation rate, i.e., the average number of mutations per gamete. In order to convert figures on the rate of mutation at specific loci to a "total" mutation rate, one must make certain assumptions concerning the number of mutable loci present in man. This is an extremely thorny problem for *Drosophila* or *Neurospora*, and even worse for man. Nor does it seem that here we can anticipate rapid advances with respect to human material. For the present, human genetics, in problems involving gene number, will have to be guided by the findings in other animals. Fortunately, there is an approach to the question of "total" mutation rates which does not involve gene number per se. Recently, Morton, Crow, and Muller[11] have described a mathematical formulation which enables one to estimate the total mutation rate per gamete per generation on the basis of a comparison of certain attributes of the offspring born to consanguineous as contrasted to nonconsanguineous marriages. This is a technique which should be quite useful. Unfortunately, the data now available which could be fed into these equations are of limited validity. This is a situation which time will certainly rectify, since there is currently a great deal of interest all over the world in the outcome of consanguineous marriage.

There is one final point I should like to make concerning studies of spontaneous mutation rates. It is a well-established fact that mutations with very minor phenotypic effects far exceed in number the mutations with major effects, with which it is usually convenient to work in experimental material. Better techniques for evaluating both the frequency and the effects of these "small mutations" are urgently needed. A recent development in human genetics may point the way to approaching these small mutations. It now appears that the distinguishing feature between sickle cell and normal hemoglobin may be a simple amino acid substitution in a polypeptide chain.[12] This difference is determined by a single gene substitution. One may speculate that the rather marked effect on the properties of hemoglobin induced by this gene substitution in the case of sickle cell hemoglobin are due to the fact that this particular amino acid substitution is especially critical. The techniques are now available for analyzing the hemoglobin of different individuals, literally amino acid by amino acid. Such techniques promise to give much needed insight into the amount of concealed genetic variability in protein structure and, by inference, into the frequency of undetected mutation. Such mutation may be of importance either through a cumulative effect or as supplying a significant source of variation when environmental conditions change.

The Induced Mutation Rate per Locus per Unit of Ionizing Radiation. The difficulties in reaching conclusions concerning the sensitivity of human genes to ionizing radiation are readily apparent. There are a number of circumstances under which substantial groups of human beings have received relatively large amounts of radiation. However, in no instance is the amount of radiation sufficiently large that one can survey the population involved with respect to the rate of

appearance of specific new mutant phenotypes, with any reasonable expectation of detecting a difference between the progeny of irradiated and controls. Rather, the approach must be much less specific, involving changes in the vital statistics of the progeny, i.e., such end points as sex ratio, stillbirth and neonatal death rates, frequency of occurrence of malformation, age-corrected death rates, etc. The most ambitious attempt to date to come to grips with this problem has been the Academy-sponsored study on the children of the survivors of Hiroshima and Nagasaki.[13] This study was inconclusive, in the sense that while no clear-cut genetic effect of the atomic bombings could be demonstrated, neither, because of the magnitude of the "observational error," could effects of an extent comparable to those produced by the irradiation of experimental material be excluded. There is, however, a more positive and sometimes overlooked aspect of these studies. They permit us to set upper limits to the sensitivity of human genes to irradiation. Thus, one of the important developments in radiation genetics during the past decade has been the demonstration by Russell[14, 15] that the mutation yield per unit of radiation was some ten times greater for the mouse—the first mammal to be so studied—than for the fruit fly. It was conceivable that man was in turn conspicuously more sensitive to the mutagenic effects of radiation than the mouse. We believe that the findings in Japan tend to exclude this possibility. Other positive aspects of the Japanese studies are that they have been collected and recorded in such a fashion that the findings may for analytic purposes be combined with such other sets of pertinent data as may some day become available and that they yield normative data concerning such questions as the outcome of consanguineous marriage, which contribute to a better understanding of the population genetics of man.

Recent developments in tissue culture[16] hold real promise of a somewhat more direct approach to this question of the sensitivity of human genes to irradiation, albeit in somatic rather than in germinal cells. It is now possible to establish "clones" of tissue culture cells, all the cells in a given culture having been derived from a single ancestor cell. It thus becomes possible to irradiate suspensions of cells and then "plate them out," as it were, much as can be done with bacterial suspensions. Mutant clones can be detected by morphological or antigenic criteria, and mutation rates thus established.

The Accumulation Factor. The term "accumulation factor" has been used to designate the ratio of the number of deleterious genes which, on the average, are already present in the individual's gene complex to the number arising each generation in consequence of mutation. This figure serves as an important yardstick, both in estimating the strength of selective factors in the past and in providing a background against which increases in mutation rate must be viewed. One way of getting at this problem is the study of both the rate of death in, and the physical and mental characteristics of, the children resulting from consanguineous marriage. Böök[17] has recently presented the first unbiased, unselected, and carefully controlled data on this point; his limited material suggests that there is an average of a *minimum* of three detrimental autosomal recessive genes in heterozygous form per individual. On theoretical grounds, the true figure has been estimated to be considerably higher.[18–20] As noted earlier, there is much current interest in this topic, and important data will almost certainly be forthcoming in the near future.

A second approach to this problem consists in the methodical collection of data concerning the frequency of inherited diseases. An estimate obtained from such data will of course be a minimum estimate. At the present time, our information concerning the frequency of most inherited diseases is so poor as to make frequency estimates of this type of relatively little value. This is a situation which can certainly be rectified, although for many years in the future this approach will yield very *minimum* estimates. A third means of estimating the accumulation factor consists of the development of clinical techniques which will make it possible to detect gene effects which have in the past gone unnoted. There is increasing evidence that many so-called "recessive" genes have some manifestation when heterozygous. Individuals who possess undesirable genes which find no or very slight expression are sometimes designated as "genetic carriers." We are now able to detect, with greater or lesser degrees of accuracy, several dozen different carrier states (review in Falls and Neel[21]). Some of the most pertinent examples in this respect are drawn from the field of hematology, where the relative ease with which hematopoietic tissue and its products may be sampled has led to relatively rapid advances. While it is certainly not imminent that by subjecting an individual to a battery of tests we can come up with a precise statement concerning the number of "undesirable" genes he carries, it should at least be possible to evaluate the situation as regards quite a number of specific possibilities in the near future.

The Manner of Action of Natural Selection in Past and Contemporary Human Populations. Of all the gaps in the background knowledge necessary to a precise evaluation of the genetic risks of ionizing radiation, that gap which will some day be filled by information concerning the manner of action of natural selection looms in my mind the largest. Many current treatments of the problem assume that the combined efforts of the physician and the sanitary engineer, coupled with agricultural and transportation developments that render famine unlikely, have brought the processes of natural selection which have shaped the human race to a virtual standstill. It is difficult to discern a really sound basis for this rather sweeping opinion, since we know so little about the workings of natural selection in either primitive or contemporary populations. There can be no question of some important recent shifts as regards selective pressures. But whether these shifts are of a magnitude or type which actually mean that the human species is no longer capable of evolving to meet rapidly changing conditions, as some would maintain, is to me a very moot point indeed. There appear to be many opportunities to study natural selection at work today.[22] These range from studies on causes of individual fertility differences to the relationship between diet and health. Recent developments with respect to the abnormal human hemoglobins have provided a striking example of how the frequency of an apparently undesirable trait can be maintained through the mechanisms of balanced polymorphism. It is of the utmost importance to determine the degree to which the human species may be "buffered" against adverse genetic developments by such genetic systems.

Mathematical Models. Having discussed the principal types of information we need in order to evaluate the genetic risks of increasing exposure to ionizing radiation, I do not want to leave you with the impression that, if this information were available, we would have only to feed it into certain equations and the answers would emerge like sausages from the other end. Despite the brilliant contributions

of Wright, Fisher, Haldane, and, more recently, Kimura, to the mathematical theory of evolution, this subject is still in its early stages. Most statistical models in this field have dealt with events at a single locus represented in a population by two alleles, with one of the alleles bearing a fixed selective relationship to the other, or with a relatively simple departure from this situation. In truth, of course, genetic changes in populations in consequence of ionizing radiation are the result of events at many thousands of loci, each represented by multiple alleles, with the selective value of a particular gene a function of the genetic makeup of the organism at many other loci. As the fifth general development necessary to an evaluation of this problem, then, I would mention the development of a mathematical approach which goes far beyond present techniques in recognizing the complexity of the problem and which is evolved at all times to take advantage of present-day electronic computers.

Concluding Remarks. In concluding this very brief survey of some of the problems of human genetics, I would like to face squarely one practical issue. The most precious asset of mankind today is the germ plasm which has enabled him to develop the culture we all enjoy. If current developments—of which increasing exposure to ionizing radiation is only one—threaten the integrity of this germ plasm and are paving the way for the decline of our species, then here is a problem equal in magnitude to any with which we are currently confronted. I think it important to emphasize that the kind of data necessary to an intelligent evaluation of the problem will not issue from the investigations of a few scattered geneticists but will require a greatly expanded effort on both human and nonhuman material, involving many teams of highly trained personnel. The annual financial support of research in human genetics in this country today is probably well below the cost of a single large jet bomber. While I by no means believe that genetic disaster is just around the corner, there is no doubt that the problems with which we are confronted call for an order of magnitude of research effort far in excess of what in the past, in the field of biology, has seemed adequate.

* Contribution to the Symposium on Genetics and Radiation Hazards of the National Academy of Sciences, under the chairmanship of G. W. Beadle.

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*THE SCOPE OF GENETICS**

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"The experiments which will here be discussed took their origin from artificial fertilisations which have been carried out on ornamental plants in order to obtain new color variants." This opening sentence of Gregor Mendel's paper points to one of the humble origins of genetics: horticulture. Significantly, as late as 1901, it was the Royal Horticultural Society of London that sponsored the English translation of the then newly rediscovered classic. But the practical desires of the lovers of flowers were not the only incentives to the birth of genetics. All through the eighteenth and nineteenth centuries the species problem occupied the minds of biologists. On the one hand, Linnaeus had defined species in a way which lacked operational usefulness: There are as many species as there were diverse forms created at the beginning by the Infinite Being. On the other hand, Linnaeus and other plant hybridizers attempted to probe into the nature of species by experimental means. Were hybridizations between different species possible? Could they be induced artificially? Did they ever occur in nature? What kind of organism was a species hybrid? Was it sterile like the mule? If fertile, what kind of offspring did it produce?

In 1859, Darwin's *Origin of Species* proved to be the successful break through the confines of a static conception of species within which biology had accumulated so much of its unordered bulk of empirical facts. Then plant hybridization became a potential tool for progress in a new range. Mendel himself, who began his studies before the appearance of Darwin's book but wrote his report six years after that event, by then had recognized clearly the role of genetics in this respect. The detailed analysis of successive generations of hybrids "seems to be the only right way," he wrote, "by which we can finally reach the solution of a question the importance of which should not be underestimated in connection with the history of the evolution of organic forms."[1]

Genetics and evolution were indeed to become intimately and fruitfully associated with one another; but the scope of genetics was destined to embrace many other regions. From variations in color and form, and from the species problem and hybridization, the emphasis soon shifted to the permanent units which underlie the externally visible phenomena. Mendel's essential discovery had been the

recognition that the hereditary contributions of two parents were not each undefined wholes which in the offspring mingled into a new whole. On the contrary, he showed that the hereditary makeup consists of many separable elements which recombine in many ways in the sequences of generations and always reappear in unchanged nature. Bateson called these units factors, "makers." Johannsen, in 1909, coined the term "gene." For him, "the word gene was . . . completely free from any hypothesis." It was meant "solely to express the fact that characters of the organism are conditioned by special, at least partially separable and thus in a sense independent 'states,' 'factors,' 'units' or 'elements' in the constitution of the germcells."

Johannsen's reluctance to go beyond the statements that "we can only operate with the knowledge that such constitutional elements exist" and that "no concept concerning the nature of genes is at present sufficiently well founded" was already somewhat out of date when he formulated these statements. While only the past few years have led to specific insights into the nature of the genetic material, the stage was set in 1900 to give the Mendelian gene its material counterpart as part of visible elements of cells, namely, the chromosomes. Roux and Weismann[2] had prophetically speculated on the nature of the chromosomes as bearers of hereditary material. Sutton and Boveri showed how the specific findings of the Mendelians fitted in with the specific observations of the cytologists. Assuming that the chromosomes carry the genes, the demonstrable distribution of the chromosomes in successive generations accounts for the Mendelian laws.

The chromosomes are limited in numbers. Few organisms contain as many as a hundred in their nuclei, and most organisms possess many fewer chromosomes. The numbers of genes are larger than those of the chromosomes. Thus the separateness of the chromosomes is not sufficient to account for the separateness of genes. This was foreseen early by Boveri but was established only later, by Morgan and Sturtevant. Each chromosome is a linear assemblage of numerous genes whose separability by recombination depends on physical exchanges of parts between chromosomes.

The Mendelian discovery that the hereditary material of an organism is not a homogeneous mass but is subdivisible into genes has often been compared to the discovery of atoms. There is, however, a striking difference in the way genes and atoms can be handled by the experimenter. The chemist or physicist can actually isolate his elementary particles, singly or en masse, and study their properties by themselves. The geneticist can do this only very incompletely. Each gene is part of a complex interacting system. A gene becomes meaningful as a biological unit in terms of a co-operating, not an isolated, material. The geneticist corresponds to a chemist whose knowledge of the properties of hydrogen and oxygen atoms would be based solely on the role they play within protein molecules!

Great progress has been made during the last decade in defining the genic material in chemical terms. The essential role of nucleic acid has been established. The transformation phenomena in the *Pneumococcus* micro-organisms in which extracts of deoxyribosenucleic acid (DNA) gained from one strain can transmit specific genic properties to another strain have given us genes in test tubes. The chemistry of DNA is complex, but it is chemistry. The test-tube genes can be changed by radiation and chemical agents: they can be made to mutate. Some day we

will be able to define in physicochemical terms the difference between DNA configurations which may cause redness of a flower or whiteness, well-functioning of a brain or idiocy. Clearly the gene is no longer Johannsen's materially undefined element. But to the biologist the DNA in the test tube is of transitory interest only. Did he not know of its role in the cell—before the cell had released it or after the cell had again received it—it would be of little concern to him.

"The central problem of biology is the physical nature of living substance. It is this that gives drive and zest to the study of the gene." Thus wrote L. Stadler in 1954, shortly before his death. Genetics has come close to the solution of this problem. Fortunately, the scope of genetics transcends by far the problem as defined by Stadler. He himself would have persisted forever in the search for still deeper insights. The central problem of biology, if there is one single one, is indeed inseparably bound up with the physical nature of living substance; but this problem is on a level different from that of the physical nature of the gene. The central problem of biology has often been hinted at: the organization of physical matter to form an organism, the emergence of biological systems, of co-ordinated cellular activities, of development, behavior, evolution.

Organization and emergence are words which admittedly do not lead to analysis. They would not have given us the knowledge of the DNA gene. But they are concepts in their own right, with their own usefulness. They call us back to the organism, which is the object of our study as biologists. We rejoice in the success of our analyses of parts, but the facts found are biologically meaningful only within the realization of their role in the whole. When the physical nature of the material basis of a living system has become completely known and the dynamics of the biochemical interactions within it can be followed in all their individual steps, chains, and cycles, the integrated nature of these processes, the possibility of their existence, and the history of their assembly will still be a cause of wonder.

The scope of genetics as a central biological discipline rests on the fact that genes are those parts of biological systems which give continuity to them. The primitive experiment of cutting an amoeba in two and finding that only the nucleated part survives and multiplies has been refined until we know that it is the genic material on which survival and multiplication of the organism depends. Most of the cellular material may be removed without permanent harm. Regeneration soon takes care of replacement. A gene, however, once removed from a cell, is not regenerated. Within a cell, a gene always comes from a gene. Again, this attribute of the gene is part of the organization of the whole. The material structure of the gene seems to carry within it the potentiality of its replication, but actual genic multiplication depends on the presence of a whole assembly of structures and processes.

The discovery of the cyclical nature of many biochemical sequences in cellular metabolism has been one of the significant events of our times. Genes are agents in these cycles not only because they determine the existence of enzymes, without which the reactions would not proceed sufficiently, but because some of these reactions themselves must lead to the making of more genes. We admire a jongleur who tosses and keeps rotating several rings moving in space. Figuratively speaking, a cell consists of thousands of such rings functionally bound together not by an outside mover but by themselves. The rings are not all of equal rank. Destroy some, and the rest will re-create them. Destroy a single agent in others, and the

whole system will disintegrate. These single agents are the genes. Through them, cellular physiology is brought within the scope of genetics. Rightly, more than thirty years ago, Muller called the gene "the basis of life."

Development is of the essence of most living forms. The emergence of a multicellular organism from a single cell is one of the miracles which daily occur around us. The embryologist has studied this miracle by observation and experiment. Strangely enough, for a long time embryology and genetics remained separate in methods and concepts. The embryologist was interested primarily in those basic developmental processes which occur alike in all individuals of a species, while the geneticist was concerned with differences among individuals. Moreover, in an opposite way, another alternative between similarity and difference found the two disciplines without common ground. Embryology is confronted with differentiation, the origin of unlikeness of tissues and organs, while genetics has postulated a genic identity of the cell nuclei in organs as different as skin, brain, and intestines.

The gap between the two disciplines is being narrowed at present. It has always been obvious that genes participate in developmental determination. How else would they exert their influence, by means of which we discover genes for growth and form? More specifically, developmental geneticists have studied the embryological pathways along which different genes produce different end results. Starting from another area, the embryologists are in the process of resolving the macroscopic and microscopic events of development into their constituent biochemical reactions. Embryology becomes an aspect of cellular physiology—and the role of genes in cellular physiology thus includes their role in processes which underlie growth and development.

Even the paradox of the postulated uniform genetic constitution of cells notwithstanding their differentiation is being resolved. Initial regional differences in a developing system, as in an egg, have long been known. They are consequences of such processes as diffusion of nutritive materials into the cell and of metabolic gradients. These general differences serve as evocators of specific gene actions, so that certain genes come to control one type of differentiation in one part of the embryo and other genes another type of differentiation somewhere else. Genes are part of the specificity of developmental phenomena. Genes determine whether a brain will develop here and fail to do so there, whether a wing will form or a leg, an eye or an antenna. The scope of genetics reaches to the problems of growth, form, and their origin.

Evolution early was close to the mind of the geneticist. Its study is now unthinkable without the framework of genetics. The differences between species are largely if not exclusively due to differences in their genes. Evolution is based on mutability, the origin of new varieties of genes, on the distribution and recombinations of these varieties within the members of populations, and on the effects which these diverse genotypes have on the fitness of individuals and of populations as whole entities. The New Systematics and the Modern Synthesis in evolution, to borrow phrases from Julian Huxley, are witness to the part which genetics has taken in the transformation of these subjects.

Evolution is a historical field. Just as the history of the earth, or that of astronomical systems, is based on the properties of elementary physical particles, so the history of life on our planet is based on the properties of its genic material. The

scope of physics and chemistry extends to astronomy and geology without depriving these sciences of their own spheres of endeavor. To say—as it has been said—that a lion or a palm is only some genes' way of making more genes expresses deep insights which link the lion and the palm to genetics—although little work on the genetics of lions or palms has been carried out. But the lions and palms remain valid topics for biological study as lions and palms. How evolution brought forth these specific species is not explained simply by referring to their genes.

The analysis of animal behavior has rightly been re-recognized as another central discipline within biology. The inheritance of behavior patterns and the inheritance of plasticity in the range of possible behavioral phenomena are being rediscovered in general and analyzed in detail. Genes determine the degree of exploratory behavior in rats, intelligence or dullness in running through a maze, fear reactions in birds to an object resembling in outline a specific predator, or the details of a complex song. This is amazing and intelligible at the same time. If chemicals can change intimately one's reactions, if under the influence of drugs spiders change the pattern according to which they weave their webs, why should not genes as biochemical cellular agents do the same? If genes can help to organize the differential development of a complex nervous system, why should they not be able to incorporate in it the subtleties of inborn reactions? The wonder is that there *is* behavior. Genetics provides one of the analytical tools for its study.

The scope of genetics, with its enlightenment of all branches of biology, makes it an obvious partner in the pursuit of applied biology. From horticulture to plant and animal breeding in general, the concepts and methods of genetics—be it individual or strain genetics in terms of hybridization and segregation, or population genetics in terms of equilibria and selection—form indispensable parts of procedure. Problems of pest control involve the existence of innately sensitive strains and the origin of resistant ones. Host-parasite relations depend on a balance of genetically determined interactions between the two components of the combating teams.

Human genetics is both a basic and an applied science. Knowledge of the genetic makeup of the species *Homo sapiens*, in its individuals and varieties, is the latest gain in the long history in which anatomy and physiology, in ever more penetrating modes, have provided insight into the biological nature of Man and anthropology into that of men. Eugenics, the planning toward rational improvement of the genetic makeup of human populations, has been regarded as a utopian dream, and a dangerous one in addition. Many of our concerns have been recognized as being excessive or even unnecessary, and our eagerness to act precipitously has rightly decreased. But, while the attempt at improvement beyond the present average could well be relegated to the future, even the most determined opponents of positive eugenics have now realized with the fervor of converts that negative eugenics, the attempt to inhibit the increase of harmful genotypes, is an immediate necessity.

In the affairs of mankind, as a community of explorers and of transformers, genetics has indeed come to occupy a significant place.

* Presented as the introduction to a Symposium on Genetics held under the chairmanship of G. W. Beadle at the ninety-fourth annual meeting of the National Academy of Sciences in Washington, D.C., April 15, 1957.

[1] It is amusing to note that the English translation, made under Bateson's influence, changed Mendel's careful wording "nicht zu unterschätzen" to the overstatement "cannot be overestimated."

[2] The name of August Weismann has been repeatedly misspelled in recent years. It should suggest a wise man, not a white man.

DYNAMIC PROGRAMMING AND STATISTICAL COMMUNICATION THEORY

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1. *Introduction.*—The purpose of this paper is to present some applications of the functional equation technique of dynamic programming[1, 2] to the study of some multistage stochastic decision processes arising in statistical communication theory.

Our starting point is a paper by Kelly[3] in which it is shown that the rate of transmission, as obtained by Shannon from considerations of coding of information,[4] can be obtained from a certain multistage process with a suitable criterion function. In this paper we shall complete a result of Kelly's and considerably extend the scope of the investigation. Further results and proofs of the theorems stated below will be presented in a subsequent publication.

2. *An M-Signal Noisy Channel.*—Consider a noisy channel which is called upon to transmit any of M different signals, which we name 1, 2, . . . , M, in succession. Let

p_{ij} = the conditional probability that the j signal has actually been sent whenever the i signal is received;

q_i = the probability that the i signal is received at any particular time.

A gambler, upon receiving a particular signal, is required to place bets on what he believes the transmitted signal to have been. He is allowed to bet a quantity z_i that the i signal was transmitted, subject to the restrictions that $\sum_i z_i \leq x$, his initial capital, and $z_i \geq 0$. If he bets correctly, he then receives $r_i z_i$, otherwise nothing. This process continues for N stages, with a payoff at the end of each stage. Assuming that the transmitted signals are independent of each other and that the gambler wishes to maximize the expected value of a function $\phi(w)$ of the final total at the end of the process, the problem is to determine an optimal wagering policy.

3. *Dynamic Programming Formulation.*—Let us define the sequence of functions

$$f_N(x) = \text{the expected value of } \phi(w) \text{ obtained using an optimal } N\text{-stage wagering policy, starting with a capital of } x. \tag{3.1}$$

for $N = 1, 2, \ldots$, and $x \geq 0$.

Then the principle of optimality yields the recurrence relations

$$f_1(x) = \sum_{i=1}^{M} q_i \left\{ \operatorname*{Max}_{\sum z_i \leq x} \sum_{j=1}^{M} p_{ij}\, \phi\left(r_j z_j + x - \sum_{s=1}^{M} z_s \right) \right\},$$

$$f_N(x) = \sum_{i=1}^{M} q_i \left\{ \operatorname*{Max}_{\sum z_i \leq x} \sum_{j=1}^{M} p_{ij}\, f_{N-1}\left(r_j z_j + x - \sum_{s=1}^{M} z_s \right) \right\}, \quad N \geq 2. \tag{3.2}$$

If we specialize the function $\phi(w)$, we obtain a noteworthy result:

THEOREM 1. *In the case where* $\phi(w) = \log w$, *we have*

$$f_N(x) = \log x + Nk, \tag{3.3}$$

where

$$k = \sum_{i=1}^{M} q_i \left\{ \operatorname*{Max}_{\sum z_i \leq 1} \sum_{j=1}^{M} p_{ij} \log \left(r_j z_j + 1 - \sum_{1=s}^{M} z_s \right) \right\}. \tag{3.4}$$

The optimal policy is independent of the number of stages remaining, independent of the quantity of money available, and independent of the sequence $\{q_i\}$. *It is determined by the maximization in equation* (3.4).

A particular case of the above in which it is required that $\sum_i z_i = x$ yields the expression $-\sum_i p_i \log p_i$ of Shannon. This furnishes an interesting link with information theory. The foregoing result resolves a problem left open by Kelly.

There is an analogous result if $\phi(w) = w^a$, $a > 0$.

4. *Generalizations.*—The results of the preceding section may be generalized in many directions, in particular, to time-dependent processes and to the case where there is a continuum of types of signals. In both cases the functional equation technique is applicable, and the analogue of Theorem 1 holds.

Another interesting type of process to consider is that in which the p_{ij} are fixed, but unknown, constants. For an expository account of the problems encountered in this area we refer to Robbins;[5] cf. also Bellman[6] and Robbins.[7]

As we shall show in a subsequent paper, a number of these problems may be treated by means of the foregoing techniques.

5. *Correlated Signals.*—Let us now consider the case where the signals are not independent. Although a large variety of questions of this type may be formulated, the following discussion of a simple process will illustrate the general method that may be employed.

Assume that there are only two types of signals, say 0 and 1, that the probability of correct transmission at any stage depends upon whether or not the preceding signal was transmitted correctly, and that the gambler bets at each stage a certain quantity of his resources that the signal he receives was actually sent. Let

$$\begin{aligned} p_k &= \text{the probability of correct transmission of the } k\text{th signal if the } (k-1)\text{st signal was transmitted correctly.} \\ r_k &= \text{the probability of correct transmission of the } k\text{th signal if the } (k-1)\text{st signal was transmitted incorrectly.} \end{aligned} \tag{5.1}$$

Define the sequence of functions

$$f_k(x) = \text{the expected value of the logarithm of the final capital obtained from the remaining } k \text{ stages of the original } N\text{-stage}$$

process, when one has a capital of x and the information that the $N - k$th signal was transmitted correctly, and uses an optimal policy.

$g_k(x)$ = the corresponding expected value in the case where the $N - k$th signal was transmitted incorrectly. (5.2)

Then

$$f_k(x) = \operatorname*{Max}_{0 \le y \le x} [p_{N-k+1} f_{k-1}(x + y) + (1 - p_{N-k+1}) g_{k-1}(x - y)],$$

$$g_k(x) = \operatorname*{Max}_{0 \le y \le x} [r_{N-k+1} f_{k-1}(x + y) + (1 - r_{N-k+1}) g_{k-1}(x - y)]. \quad (5.3)$$

It follows inductively that

$$f_k(x) = \log x + a_k, \qquad g_k(x) = \log x + b_k, \quad (5.4)$$

where a_k and b_k are independent of x. The recurrence relations for the sequence $\{a_k, b_k\}$ are readily obtained from equation (5.3).

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*SOLUTION OF THE BURNSIDE PROBLEM FOR EXPONENT 6**

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The restricted Burnside problem for exponent 6 was solved by Philip Hall and Graham Higman.[1] They even found the order of the largest finite group of exponent 6 generated by k elements, this order being

$$2^a 3^{b + \binom{b}{2} + \binom{b}{3}},$$

where $a = 1 + (k - 1)\, 3^{k + \binom{k}{2} + \binom{k}{3}}$ and $b = 1 + (k - 1)2^k$. A proof is sketched here that a finitely generated group of exponent 6 is necessarily finite solving the Burnside problem for exponent 6. The proof will be published in detail elsewhere.

It has been shown by Levi and van der Waerden[2] that there is a group $B(3, k)$ generated by k elements and of exponent 3 whose order is 3^K, $K = k + \binom{k}{2} + \binom{k}{3}$,

and that every other group of exponent 3 with k generators is a homomorphic image of $B(3, k)$. The following relations hold on commutators in a group of exponent 3.

$$\begin{gathered}(x, y^{-1}) = (x, y)^{-1} \\ (x, y, z) = (y, z, x) = (z, x, y), \\ (x, y, z, w) = 1.\end{gathered} \tag{1}$$

It is well known that a group of exponent 2 is Abelian, since, from $a^2 = 1$, $b^2 = 1$, $(ba)^2 = 1$, we find $ba = (ba)^{-1} = a^{-1}b^{-1} = ab$.

THEOREM. *A group G of exponent 6 generated by a finite number k of elements is finite.*

LEMMA 1. *The cubes of the elements of G generate a normal subgroup M of index of most 3^K, $K = k + \binom{k}{2} + \binom{k}{3}$.*

This follows directly from the result of Levi and van der Waerden.

LEMMA 2. *M is generated by a finite number of elements of order 2. The derived group M' of M is of index a power of 2 in M, and M' is generated by a finite number of elements of the form abab, where $a^2 = 1$, $b^2 = 1$.*

M, being of finite index in a finitely generated group, is itself finitely generated, say by $\alpha_1, \ldots, \alpha_m$. M is also generated by elements of order 2, namely, the cubes of elements of G. Thus each α can be expressed in terms of a finite number of elements of order 2, and the finite number of elements of order 2 needed to express $\alpha_1, \ldots, \alpha_m$ will be a set of generators for M. If M is generated by $x_1, \ldots, x_t$ with $x^2 = 1$, $i = 1, \ldots, t$, then M' is of index at most 2^t in M. Also, M' is generated by the commutators $x_i^{-1}x_j^{-1}x_ix_j$ and their conjugates, and so by a finite set of these. Hence M' is generated by a finite number of elements of the form abab, where $a^2 = 1$, $b^2 = 1$.

The rest of the proof is devoted to showing that M' is of exponent 3 and hence finite from the result of Levi and van der Waerden, and even from the less precise result of Burnside's original paper.[3] Since M' is of finite indes in G, the finiteness of G will follow from the finiteness of M', providing the theorem.

LEMMA 3. *If H is generated by $x_1, \ldots, x_n$ and if every subgroup of H generated by four of the x's is of exponent 3, then H is of exponent 3.*

Here we may take H as generated by $x_1, \ldots, x_n$, with defining relations $z^3 = 1$ for every z in a subgroup of H generated by four x's. Then $B(3, n)$ is a homomorphic image of H. Philip Hall's collecting process applied to words in $x_1, \ldots, x_n$, together with the validity of relations (1) whenever x, y, z, w are taken from the set of x's and their inverses, will show that the order of H is at most that of $B(3, n)$. Hence H is indeed $B(3, n)$, and H is of exponent 3.

LEMMA 4. *If $H = \{x_1, x_2, x_3, x_4\}$ is of exponent 6 and if each of the subgroups generated by three of the x's is of exponent 3, then H is of exponent 3.*

This is proved by calculations using relations such as $((x_1, x_2)x_3x_4)^6 = 1$, together with the relations on three of the x's.

LEMMA 5. *If $H = \{x, a, b\}$ is of exponent 6 and if $x^2 = 1$, $a^3 = 1$, $b^3 = 1$, $xax = a^{-1}$, $xbx = b^{-1}$, then $\{a, b\}$ is of exponent 3.*

This is the most difficult lemma of all and involves some complicated calculations with relations.

LEMMA 6. *If $H = \{x, a, b\}$ is of exponent 6 and if $x^2 = 1$, $a^3 = 1$, $b^3 = 1$, $xax = a^{-1}$, $xbx = b$, then $\{a, b\}$ is of exponent 3.*

Since $x(bab^{-1})x = (bab^{-1})^{-1}$ and $x(b^{-1}ab)x = (b^{-1}ab)^{-1}$, it follows from Lemma 5 that $\{a, bab^{-1}\}$ and $\{a, b^{-1}ab\}$ are of exponent 3. With these facts and further relations the lemma follows.

LEMMA 7. *If* $H = \{x, a, b, c\}$ *is of exponent 6 and if* $x^2 = 1$, $a^3 = b^3 = c^3 = 1$, $xax = a^{-1}$, $xbx = b^{-1}$, $xcx = c^{-1}$, *then* $\{a, b, c\}$ *is of exponent 3.*

From Lemma 5 it follows that $\{a, b\}$, $\{a, c\}$, and $\{b, c\}$ are of exponent 3. Thus $x(a, b)x = (a^{-1}, b^{-1}) = (a, b)$. Hence from Lemma 6 $\{(a, b), c\}$ *is of exponent 3.* Also, $x(aba)x = (aba)^{-1}$, whence, from Lemma 5, $\{aba, c\}$ is of exponent 3. From these and similar relations the lemma follows.

LEMMA 8. *If* $x^2 = 1$, $a_i^3 = 1$, $i = 1, \ldots, n$, $xa_ix = a_i^{-1}$, *and if* $\{x, a_1, \ldots, a_n\}$ *is of exponent 6, then* $\{a_1, \ldots, a_n\}$ *is of exponent 3.*

This is a direct consequence of Lemmas 3, 4, and 7.

LEMMA 9. *If* $H = \{a, b, c\}$ *is of exponent 6 and* $a^2 = b^2 = c^2 = 1$, *then* H' *is of exponent 3.*

We easily find that H′ is generated by the five elements

$$\alpha_1 = abab, \qquad \alpha_4 = abcacb,$$
$$\alpha_2 = acac, \qquad \alpha_5 = abcbca,$$
$$\alpha_3 = bcbc,$$

and then show that the elements α_i, $i = 1, 2, 4$, $\alpha_3\alpha_5^{-1}$, $\alpha_3^{-1}\alpha_5$, $\alpha_3\alpha_i\alpha_5^{-1}$, $\alpha_2^{-1}\alpha_i\alpha_5$, $i = 1, 2, 4$, are transformed into their inverses by the element a and that they are of order 3. By Lemma 8 these elements generate a group K of exponent 3. But K is normal of index 3 in H'. Hence H' is of exponent 9 and so of exponent 3.

LEMMA 10. *If* $a^2 = b^2 = c^2 = d^2 = 1$ *and if* $H = \{a, b, c, d\}$ *is of exponent 6 and* $\alpha = abab$, $\beta = cdcd$, *then* $T = \{\alpha, \beta\}$ *is of exponent 3.*

Write $\beta_1 = cdcd$, $\beta_2 = acdcda$. Then, by Lemma 9, $\{\beta_1, \beta_2\}$ is of exponent 3. We show that each of α, $\beta_1\beta_2^{-1}$, $\beta_1^{-1}\beta_2$, $\beta_1\alpha\beta_2^{-1}$, $\beta_1^{-1}\alpha\beta_2$ is transformed into its inverse by the element a and is of order 3. By Lemma 8 they generate a group U of exponent 3. U is normal of index 3 in $V = \{\alpha, \beta_1, \beta_2\}$, whence V is of exponent 9 and so is of exponent 3. Hence T as a subgroup of V is of exponent 3.

LEMMA 11. *If* $a^2 = b^2 = c^2 = d^2 = e^2 = f^2 = 1$ *and if* $H = \{a, b, c, d, e, f\}$ *is of exponent 6 and* $\alpha = abab$, $\beta = cdcd$, $\gamma = efer$, *then* $T = \{\alpha, \beta, \gamma\}$ *is of exponent 3.*

Write $\beta = \beta_1 = cdcd$, $\beta_2 = acdcda$, $\gamma = \gamma_1 = efef$, $\gamma_2 = aefefa$. Then, by Lemma 9, $\{\beta_1, \beta_2\}$ and $\{\gamma_1, \gamma_2\}$ are of exponent 3. We show that α, $W(\beta_1, \gamma_1)\alpha W(\beta_2, \gamma_2)^{-1}$, and $W(\beta_1, \gamma_1)W(\beta_2, \gamma_2)^{-1}$, where $W(u, v)$ is any word in two generators u and v, are transformed into their inverses by the element a and are of order 3. By Lemma 8, they generate a group U of exponent 3. U is normal of index 27 in $V = \{\alpha, \beta_1, \gamma_1, \beta_2, \gamma_2\}$. V/U is of exponent 3, and so V is of exponent 9 and so 3. Thus T as a subgroup of V is of exponent 3.

LEMMA 12. M' *is of exponent 3.*

This comes from Lemmas 2, 3, 4, and 11. This proves M' finite, and so the finiteness of G, which is our theorem.

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PARABOLIC EQUATIONS

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This note outlines the proofs of theorems on the continuity of solutions of linear parabolic and elliptic partial differential equations. These *a priori* continuity theorems are important for their applications to nonlinear equations. Our original motivation was the existence problems for fluid flow, especially the general compressible viscous flow, and the turbulence problem. We were led to the problem solved here by a process of reduction, isolating what seemed the essential difficulty facing an attack on the general nonlinear equations.

We study parabolic equations of the form

$$\sum_{i,j} \frac{\partial}{\partial x_i}\left[C_{ij}(x_1, x_2, \ldots, x_n, t)\frac{\partial T}{\partial x_j}\right] = \frac{\partial T}{\partial t} \qquad \text{or} \qquad \nabla\cdot[C\cdot\nabla T] = \dot{T}, \tag{1}$$

where C is a symmetric real matrix varying in space and time and constrained only by universal upper and lower bounds $c_2 \geq c_1 > 0$ on its eigenvalues. Such equations characterize diffusion or heat conduction.

THEOREM. *If $T(x, t)$ is a solution of equation (1) satisfying $|T| \leq B$ when $t = t_0$, then for $t_2 \geq t_1 \geq t_0$*

$$|T(x_1, t_1) - T(x_2, t_2)| \leq BA\left[\left(\frac{|x_1 - x_2|}{\sqrt{t_1 - t_0}}\right)^{\alpha} + \left(\frac{t_2 - t_1}{t_1 - t_0}\right)^{\alpha/(2\alpha+2)}\right], \tag{2}$$

where α and A are constants that depend only on c_1, c_2 and the space dimension n. A similar result obtains for an elliptic boundary-value problem.

THEOREM. *If $T(x)$ satisfies $\nabla\cdot(C\cdot\nabla T) = 0$ in a region and $|T| \leq B$ on the boundary, then*

$$|T(x) - T(\bar{x})| \leq BD\left[\frac{|x - \bar{x}|}{\min\,(d(x), d(\bar{x}))}\right]^{\alpha/(\alpha+1)} \tag{3}$$

where α is the α of equation (2) and D depends only on c_1, c_2 and n.

We attack via fundamental solutions, which are conveniently unified in a single *characterizing function* $S(xt\bar{x}\bar{t})$. S is defined and positive whenever $t > \bar{t}$. For fixed $\bar{x}$, $\bar{t}$ S satisfies equation (1) and has unit weight: $\int S\,dx = 1$, where dx conventionally stands for the volume element in the Euclidean space. For fixed x, t S is similarly a fundamental solution of the adjoint equation

$$\nabla_{\bar{x}}\cdot[C(\bar{x}, \bar{t})\cdot\nabla_{\bar{x}}S] = -\frac{\partial S}{\partial \bar{t}}.$$

For any solution T of equation (1) bounded at time $\bar{t}$, we have a basic representation identity for $t > \bar{t}$,

$$T(x, t) = \int S(xt\bar{x}\bar{t})\, T(\bar{x}, \bar{t})\, d\bar{x}, \tag{4}$$

and also the special case

$$S(x\bar{t}\bar{x}\bar{t}) = \int S(xt\bar{x}\bar{t})\, S(\bar{x}\bar{t}xt)\, d\bar{x}. \tag{5}$$

The inequality

$$|T(x, t) - T(x', t')| \leq \max_{\bar{x}} |T(\bar{x}, \bar{t})| \int |S(xt\bar{x}\bar{t}) - S(x't'\bar{x}\bar{t})|\, d\bar{x}, \tag{6}$$

which follows from equation (4), shows that continuity of the solution T is equivalent to L_1 functional continuity of S in the appropriate sense.

Our argument has three main stages, in which different inequalities are established. The major result of the first stage is important in itself and is used in all later stages. It controls the rate of "spreading out" of a fundamental solution and seems the key to the whole problem. We begin by considering a fundamental solution $T = S(xt00)$ with singularity at the origin. We derive, first,

$$T \leq kt^{-n/2}. \tag{7}$$

As in inequality (7), constants that depend only on c_1, c_2, and n will be indicated by the letter k. Relation (7) can be derived from equation (5) and an L_2 inequality,

$$\int T^2\, dx \leq kt^{-n/2}. \tag{8}$$

This, in turn, follows from the inequality

$$\int |\nabla T|^2\, dx \geq k[\int T^2\, dx]^{(n+2)/n}\, [\int T\, dx]^{-4/n},$$

which holds for any positive function T, and another inequality derived thus:

$$\frac{d}{dt} \int T^2\, dx = 2 \int T\dot{T}\, dx = 2 \int T\nabla \cdot [C \cdot \nabla T]\, dx$$

$$= -2 \int \nabla T \cdot C \cdot \nabla T\, dx$$

$$-\frac{d}{dt} \int T^2\, dx \geq 2c_1 \int |\nabla T|^2\, dx.$$

We define a moment M as

$$M = \int rT\, dx = \int \sqrt{x_1^2 + \ldots + x_n^2}\, T\, dx, \tag{9}$$

and the estimate to be established,

$$M \leq k\sqrt{t}, \tag{10}$$

is the first main result. We also define an "entropy" Q as

$$Q = -\int T \log T\, dx. \tag{11}$$

From relation (7) it follows that

$$Q \geq k + \left(\frac{n}{2}\right) \log t, \tag{12}$$

and from simple extremal considerations using only $T \geq 0$ and $\int T\, dx = 1$, we obtain

$$M \geq ke^{Q/n}. \tag{13}$$

Differentiating equation (11), we find

$$\begin{aligned} \dot{Q} &= -\int (1 + \log T)\dot{T}\, dx \\ &= -\int (1 + \log T)\, \nabla\cdot[C\cdot\nabla T]\, dx \\ &= \int \nabla(\log T)\cdot C\cdot\nabla T\, dx \\ &= \int \nabla(\log T)\cdot C\cdot\nabla\,(\log T)(T\, dx), \end{aligned}$$

so that

$$\begin{aligned} c_2\dot{Q} &\geq \int |C\cdot\nabla \log T|^2 (T\, dx) \\ &\geq [\int |C\cdot\nabla \log T| (T\, dx)]^2 \\ &\geq [\int |C\cdot\nabla T|\, dx]^2. \end{aligned}$$

Since, analogously, $\dot{M} = -\int \nabla r\cdot C\cdot\nabla T\, dx$, and $|\nabla r| = 1$, we have a basic fact:

$$c_2\dot{Q} \geq (\dot{M})^2. \tag{14}$$

The three inequalities (12), (13), and (14) together enable us to bound both M and Q both above and below as functions of time; in particular, we obtain relation (10).

In the second stage of our work we consider two fundamental solutions T_1 and T_2 with adjacent singularities $(x_1, 0)$ and $(x_2, 0)$. We show that there is a positive function $\epsilon(\tau, |x_1 - x_2|)$ such that

$$\int \min (T_1, T_2)\, dx \geq \epsilon(\tau, |x_1 - x_2|) > 0$$

or (15)

$$\int |T_1 - T_2|\, dx = \int [T + T_2 - 2\min (T_1, T_2)]\, dx \leq 2 - 2\epsilon(\tau, |x_1 - x_2|)$$

when $t \geq \tau$. Since the family of equations of the form (1) with C bounded as we assume is invariant under the transformation $x = \lambda x'$, $t = \lambda^2 t'$, any *a priori* bounds applying to solutions must be preserved under this transformation. This gives us a principle of dimensional analysis. In particular, the form of relation (15) must be

$$\int \min (T_1, T_2)\, dx \geq \epsilon\left(\frac{\tau}{|x_1 - x_2|^2}\right), \tag{16}$$

where the function ϵ depends only on c_1, c_2, and n.

Our proof of this depends on a study of the quantity

$$F(\delta, t) = \int \zeta^2[\log(T_1 + \delta) + \log(T_2 + \delta)]\, dx, \tag{17}$$

where ζ is a C^∞ function, $0 \leq \zeta \leq 1$, and $\zeta = 0$ outside a compact region containing the singularities of the two fundamental solutions, and δ is a small positive constant. For small δ, the following bounds on F are valid:

$$(a)\quad F \leq k \qquad \text{and} \qquad (b)\quad F \geq k \log \delta. \tag{18}$$

These depend only on equation (17) and the facts $T \geq 0$ and $\int T\, dx = 1$. If we assume, for purposes of contradiction, that $\int \min (T_1, T_2)\, dx < \epsilon$ for $t \leq \tau$, and that ϵ is sufficiently small in relation to δ, then we can show that for $t \leq \tau$

$$\dot{F} \geq kt^n(\log \delta)^2 + kt^{n/2}(\log \delta). \tag{19}$$

The derivation of relation (19) is somewhat involved; we use the differential equation and relations (7) and (10), but it requires no special insight. Now relations (18) and (19) are inconsistent if δ is small in relation to τ. Therefore, there is a lower limit on ϵ as a function of τ (referring to the hypothesis above on $\int \min(T_1, T_2)\, dx$), and since $\int \min(T_1, T_2)\, dx$ is an increasing quantity, relation (15) and therefore relation (16) are established.

The final phase of our attack obtains the inequality

$$\int |T_1 - T_2|\, dx \leq 2 - 2\epsilon\left(\frac{t}{|x_1 - x_2|^2}\right) \leq A\left(\frac{|x_1 - x_2|}{\sqrt{t}}\right)^{\alpha}, \tag{20}$$

where A and α depend only on c_1, c_2, and n. This more specific result is obtained by iterative use of the result (16). For this we use relation (16) with τ in a specific ratio to $|x_1 - x_2|^2$, so that $\epsilon(\tau/|x_1 - x_2|^2)$ has a specific value ϵ_0. Then we show in the iterative argument that there is a sequence of times $t_1, t_2, \ldots, t_n, \ldots$ such that the two fundamental solutions satisfy

$$\int |T_1 - T_2|\, dx \leq 2\left(1 - \frac{\epsilon_0}{2}\right)^n \quad \text{for} \quad t \geq t_n. \tag{21}$$

As a part of the inductive argument, we estimate a moment quantity

$$N_n = \int r|T_1 - T_2|\, dx \quad \text{at time} \quad t_n. \tag{22}$$

using relations (21) and (22) for time t_{n-1} and (4) and (10) to estimate N_n for time t_n. N_n enables us to control the time interval $t_{n-1} - t_n$ necessary for fulfilment of relation (21) for time t_{n+1}. Finally, we see that

$$t_n \leq k_1(k_2)^n, \tag{23}$$

from which, with relation (21), we obtain directly the special case of relation (2) where $t_1 = t_2$ (space continuity). Then a quite simple argument using relations (5) and (10) enables us to reduce time continuity to space continuity and obtain the general form of relation (2).

Elliptic boundary-value problems can be modeled as parabolic boundary-value problems, and we can use relations (10) and (2) to derive (3). Consider a parabolic boundary-value problem with a fixed boundary B bounding a region R and T prescribed on B for all times, thus determining T within R for all times. For such a problem there is a measure (Green's) function with properties analogous to the fundamental solutions in free space. For each point and time (x_2, t_2) in the region and previous time t_1 there is a positive measure with total weight 1 defined. It is clearer to divide this into a part $\rho(x_2, t_2)$ applying to the boundary cylinder B_{t_1, t_2} between times t_1 and t_2 and a part $\sigma(x_2, t_2, t_1)$ applying to R at time t_1 (R_{t_1}). In terms of this measure the values of T are given as follows in terms of prior values:

$$T(x_2, t_2) = \int_{B_{t_1, t_2}} T(x, t) d\rho_{(x_2, t_2)}(x, t) + \int_{R_{t_1}} T(x, t_1)\, d\sigma_{(x_2, t_2, t_1)}(x_1). \tag{24}$$

The measure satisfies an identity involving the characterizing function:

$$S(x_2t_2x_1t_1) = \int_{B_{t_1, t_2}} S(xtx_1t_1)\, d\rho_{(x_2, t_2)}(x, t) + \int_{R_{t_1}} S(xtx_1t_1)\, d\sigma_{(x_2, t_2, t_1)}(x_1). \tag{25}$$

In equation (25) the second integral can also be written as $d\sigma/dx_1$, the conversion of the measure into a function, and x_1 can be any point in space. Using equation (25) with relation (10), we control the portion of the weight of the total measure in the boundary part $d\rho$ in terms of the size of $\sqrt{t_2 - t_1}$ compared with the distance $d(x_2)$ from x_2 to B. Then we use equation (25) again to establish the approximation of $d\sigma$ to $S(x_2t_2x_1t_1)\,dx_1$. This reduces the problem of estimating the continuity of T at time t_2 to the free-space problem where relation (2) applies. The elliptic boundary-value problem corresponds to the parabolic one where everything is independent of time and a steady state obtains. The best continuity control is obtained by selecting $t_2 - t_1$ in proper relation to $|x_2 - x_2'|$, $d(x_2)$ and $d(x_2')$, x_2 and x_2' being two points in R. Proceeding crudely, we obtain relation (3).

Previously, relation (3) was known only up to two dimensions and was obtained by somewhat special methods. This limited the study of nonlinear elliptic problems to one and two dimensions. Relation (2) was, at most, known only in one space dimension. The key inequality (10) contains more strength than we have used here to derive relations (2) and (3).

In all our discussion we have ignored smoothness questions, because, in obtaining the results we obtain, the essential thing is that no *quantitative* smoothness assumptions are used, while any *qualitative* smoothness, such as C^∞, assumed for the coefficients of equation (1) is quite harmless.

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RADIOACTIVE FALLOUT

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1. INTRODUCTION

The radioactivity produced by the fission reaction changes its characteristics continuously and rapidly following the explosion of an atomic weapon, and the conditions of firing are of extreme importance in determining the rate at which the radioactivity descends to earth. As a result, there are in general three different kinds of radioactive fallout, the relative importance of which is determined by the nature of the weapon, principally its yield, and the conditions of firing. The first of these three types is the *local fallout*, which is insignificant unless the fireball touches or comes close to the ground but which, in case the fireball does touch the ground, can amount to a major fraction, in some instances as much as 80 per cent of the total debris. This type of fallout consists of radioactivity which is carried down by the larger particles. It consists largely of matter drawn up into the fireball from the surface, which is either totally or partially vaporized. Under these conditions so much matter is vaporized by virtue of the fireball's touching the ground that the particle sizes formed in the freshly cooled vapor are large.

The second and third types of radioactive fallout are world-wide in nature and consist of finer material; they are divided according to whether the material happens to lie in the lower part of the atmosphere, the troposphere, where rain and weather phenomena occur, or in the higher part of the atmosphere, the stratosphere, which is free of such precipitating mechanisms. The *tropospheric fallout* occurs in a matter of two or three weeks or a month or so. It occurs largely as a result of rain and snow, and water precipitation in general, and falls in the general latitude of the test site. The *stratospheric fallout*, in contrast, takes years. We are not completely certain, but it appears that an average time of something like ten years, or perhaps somewhat less, is a reasonable figure, and during this time the distribution becomes nearly world-wide. When the stratospheric fallout manages finally to pass into the troposphere, it is quickly removed by the same type of mechanism that brings down the world-wide tropospheric fallout, namely, rain and moisture.

The precipitating mechanisms consist in general of the collision of the tiny particles with moisture droplets in clouds, together with the interception of particles by falling raindrops. The first mechanism was recently suggested by Dr. Greenfield in connection with "Sunshine" problems. In addition to the scavenging action of rains and fogs, there is definite evidence for a considerable probability of pickup on direct contact of air with surfaces such as the leaves of grass and trees. Frequently, grasses are found to have higher strontium 90 content than would correspond to the soils in which they grow, and this is due undoubtedly to direct pickup.

The dissemination of strontium 90 and all fallout is greatly dependent upon the firing conditions. There is every evidence that important factors include not only contact of the fireball with the surface but the nature of the surface—whether it is land or water, the type of soil or the composition of the water, whether fresh or sea water. Also, the height to which the fireball rises is important—in particular, the height relative to the tropopause, the dividing layer between the troposphere and the stratosphere. Yield is the main consideration here. A rough rule is that megaton weapons push through the tropopause into the stratosphere, and kiloton weapons stay below the tropopause in the troposphere.

Thus we see immediately that kiloton weapons deposit their fission products much more quickly than do megaton weapons. Of course, this is of less importance in so far as the long-lived fission products, such as strontium 90 and cesium 137, are concerned, but it is of more importance for the shorter-lived fission products. As a general rule, an air-fired kiloton weapon will deposit its radioactive fallout in a period of between two weeks and one month, on the average, after the detonation, whereas an air-fired megaton weapon will deposit its radioactive fallout over many years—on the average, about ten years. Thus the effects which are due to the short-lived fission products are larger for a given amount of fission energy release in kiloton weapons than they are for air-fired megaton weapons. Considering the average age of the kiloton fission products to be one month, the external gamma-ray exposure from a megaton of fission fired as, say, 50 bombs of 20 kilotons each would be 30 times that for a single bomb giving one megaton of fission energy—if both were fired well up in the air. The fission products from the small bombs fired in Nevada would fall in the latitudes 10° N. to 60° N. in about one month, while the larger bomb would give fallout over essentially the whole earth in about

ten years. For strontium 90 effects there is relatively little difference per unit of fission yield, since even the residence time in the stratosphere is small compared with the 28-year half-life of radioactive strontium and the 27-year half-life of radioactive cesium, which is produced at slightly higher yield than strontium 90 and which appears to be disseminated in about the same way.

The contents of radiostrontium and radiocesium in the stratosphere are shown by direct measurement to be roughly the same, although the radiocesium is somewhat higher, possibly because of the slightly higher fission yield. The content of radiocesium in rain water is comparable to that of strontium 90. Also, the content of radiocesium in the human body as measured by Marinelli at Argonne and by Anderson and Langham at Los Alamos agrees well with the fact that it has an average residence time in the human body of about five months, as compared with many years for strontium 90. The radiocesium data are very interesting because of their bearing on the fallout dissemination mechanism and the confidence with which we can establish the probable future behavior of radioactive strontium. The data confirm previous suggestions as to the dissemination mechanism, that is, we find that radiocesium fallout, except of the local variety, is carried down very largely in the form of moisture droplets and that there is some direct pickup by leaves and grass on surfaces. It is captured and held tightly by the top two inches of most soils, so that the water which falls and runs off in the form of rivers is clean by the time it has drained a short distance through soil. All this is very similar to the radiostrontium behavior.

The plants pick the strontium 90, and radiocesium to a lesser extent, out of the soil and also off their leaves and take it into their systems. There appears to be a discrimination mechanism which operates in most plants, so that the strontium 90 content of the plant is considerably less, relative to its calcium content, than in the case of the soil. On the average, the discrimination factor between the topsoil and plants against strontium relative to calcium seems to be about 1.4. When the cows eat grass, they further discriminate by about a factor of 7 in making milk; hence there is an over-all protection factor for strontium 90 from the topsoil to milk of about 1.4×7 or 10. Also, there is a further discrimination factor against strontium relative to calcium in the human body. This factor is not known accurately but is known definitely to be at least as large as 2 and is thought possibly to be as high as 8. Research is now in progress to settle this. Therefore, there is a series of protective factors which makes the concentration of radiostrontium derived from milk relative to calcium in human bone not over one-twentieth and possibly as little as one-eightieth of that in the topsoil. Of course, it should be pointed out that a considerable part of the fallout is picked up directly on the leaves, and to this the factor of 1.4 does not apply; thus, for this fraction of the fallout, the protective factor may be reduced to 14. Since milk is the source of most of our calcium, this means that the actual ratio of radiostrontium concentration in new human bones relative to that in the topsoil should approach these numbers.

It must be realized that, although only a small part of the calcium is derived from vegetables and meat, a similar calculation must be made for this portion and the total average ratio obtained. It seems that the meat-vegetable over-all discrimination factor is about 10; if 20 per cent of the calcium is derived from such sources on the average, the average over-all factor will be between $^1/_{13}$ and $^1/_{30}$.

The experimental data on new human bone in children appear to give a smaller figure, $^1/_{60}$, as mentioned later.

A matter of importance in connection with the amount of strontium 90 which one would expect to be deposited in human bone as a result of atomic weapon detonations is the calcium concentration in the topsoil. Since calcium is so similar to strontium, it seems very likely, and the evidence confirms this, that high available calcium content of the soil will reduce the probability of strontium 90 being taken up into the plants. Of course, this probably does not have nearly as great an effect on the uptake of the material which is picked up directly on the leaves. We might expect, therefore, that soils which are particularly low in calcium might show higher strontium 90 contents for the grasses grown on them. This is, in fact, so, and sheep and goats and cattle feeding on such pasture displays a higher strontium 90 bone content.

How such calcium deficiencies in the soil should affect the strontium 90 uptake by the human population is a most important question. One sees immediately that food distribution systems are such that the food supply is derived from large areas, and that there is consequently a sharp reduction in the sensitivity of the human population to calcium deficiencies in local soils. This is brought out particularly well by the data on the radium contents of human bones and their obvious lack of strong dependence on the radium contents of local waters. But for people who consistently drink milk from cows grazing on such ground there should be definite effect on the amount of radiostrontium uptake, and the effect should be proportional to the radiostrontium content of the milk. So the question resolves itself largely into this: What are the strontium 90 contents of the foods that people in such regions actually consume? We find, on inspection of the food-eating habits and calculation of the strontium 90 intake relative to calcium, that the increase in average strontium 90 concentration of the food due to the low calcium content of the particular soils can hardly be more than fivefold for a soil calcium deficiency of fifty fold. That is, whereas normal soil carried about 20 gm. of available calcium in the top 2.5 inches, a region with soil of only 0.4 gm. per square foot would produce a human-body burden equilibrium of about five times that which the normal soil would produce.

In order to understand the hazard of radiostrontium, which is generally agreed to be the most hazardous of the long-lived fission products, we try to establish the maximum permissible concentration both for occupational workers and for the population in general. These numbers have been set at 1 microcurie and 0.1 microcurie for the standard man, respectively. That is, an occupational worker may carry 1 microcurie of strontium 90 in his body, whereas the average standard adult in the general public should not have over 0.1 microcurie of strontium 90. This last figure corresponds to a concentration of 100 micromicrocuries per gram of body calcium, or what we call 100 "Sunshine Units"; that is, 1 micromicrocurie of strontium 90 per gram of body calcium is defined as 1 Sunshine Unit.

Now we must try to see in some other way how our normal experiences can be brought to bear on the question, "How dangerous is atomic weapons testing from the point of view of radioactive fallout?" At the present time we have in our bodies about 0.1 or 0.2 Sunshine Unit, and children have about one-half of a Sunshine Unit. A little later I will speak about the question of the variation from

these average values; but, assuming at the moment that these are the values, what is the threat or the hazard from these quantities? Obviously, they are much smaller than the 100-Sunshine-Unit tolerance figure mentioned above. To obtain a comparison with normal experience, let us consider the fact that we know in a general way the magnitude of the radiation levels to which we are normally subjected by the cosmic rays, the potassium in our own bodies, and the uranium, thorium, and potassium in the ground and in our surroundings. We know that these quantities amount to something like 150 milliroentgens per year for an average person in this latitude. But we also know that there are considerable variations with conditions.

For example, a person living in a brick house may very well get 25–50 milliroentgens per year more than one living in a wooden house, because of the natural radioactivity of the bricks. It is also very well known that, whereas at sea level in this latitude the cosmic-ray dosage is 37 milliroentgens per year, at 5,000 feet altitude, as in Denver, Colorado, the dosage from cosmic rays is 60 milliroentgens per year—a difference of 23 milliroentgens per year. What is this in terms of strontium 90 body burden?

First, we must consider what part of the natural radiation, if any, is similar to the radiation of strontium 90 in biological effect, so that we can say without doubt and hesitancy that the physiological effects, whatever they are, will be the same for the same energy absorbed. Fortunately, the cosmic rays seem to fit this bill. In other words, we are at liberty to compare the cosmic-ray radiation dosages with the dosages from radiostrontium in our bone structure. The reason this is permissible is that the ionization density along the tracks of the mu-mesons which are the principal cosmic-ray components at sea level and at altitudes of 5,000 feet are nearly the same as those of the yttrium 90 beta rays, the principal radiation which radiostrontium emits; that is, radiostrontium has a radioactive daughter, yttrium 90, which emits a very energetic beta ray, and the ionization density along the track of this radiation is very similar to that of the mu-mesons of the cosmic rays and their disintegration electrons; it is generally accepted by health physicists and radiobiologists that radiations of the same ionization density have very similar, if not identical, biological effects for the same energy absorbed. The high energy of yttrium 90 gives it an average distance of penetration in tissue of 2 millimeters; hence any effect of local nonuniformity of deposition of strontium 90 in the bone is removed. The cosmic-ray exposure is, of course, uniform throughout the bone structure. Therefore, we can equate cosmic-ray dosage with strontium 90 dosage, and it is thus possible for us to say that the difference between one altitude and another is equal in effect, other effects being equal, to a certain number of Sunshine Units in bone. Now, to follow this thought through, 1 Sunshine Unit is equal to 3 milliroentgens per year. Therefore, the difference in annual cosmic-ray radiation dosage between Washington, D.C., or any place at sea level in this latitude, and Denver, Colorado, is equal to 8 Sunshine Units, that is, 16 times the present body burden of equilibrium bone or bone near equilibrium as we see it in young children who are growing now.

Therefore, we must see whether anything in our experience indicates that these differences are significant in terms of the occurrence of the principal effects expected of radiostrontium, namely, leukemia and bone cancer. Now, of course,

when one looks for such vital statistics, one finds that they are very hard to acquire. However, the National Institutes of Health of the Department of Health, Education, and Welfare have given us statistics for the occurrence of leukemia and bone cancer for the year 1947 for the three cities New Orleans, San Francisco, and Denver. They are shown in Table 1. It is clear from this table that there is

TABLE 1

OCCURRENCE OF BONE CANCER AND LEUKEMIA
(New Cases per Year per 100,000 Population)

	Bone Cancer	Leukemia
Denver	2.4	6.4
New Orleans	2.8	6.9
San Francisco	2.9	10.3

no obvious effect of altitude, and it is also clear that there are other factors which are noticeably more important than cosmic-ray dosage. Of course, there may still be a considerable effect of altitude hidden in large fluctuations caused by other factors which presumably are largely unknown, and we cannot say that this *proves* anything. It does, however, give us some assurance from normal experience that the effect of 8 Sunshine Units will not cause a detectable increase in bone cancer or leukemia.

This fits well with the laboratory data on animals and the limited experience on humans with radium. That is, 1 microcurie, being 1,000 Sunshine Units, is still considered to be pretty safe on the basis of the laboratory data. It is set as a tolerance for occupational workers, and it is therefore reasonable that 8 Sunshine Units should give an effect so small as to be very, very difficult to detect. It is, I think, helpful for us, however, to realize that the present body burden of strontium 90 in new bone from the weapons tests that have occurred in the past is equal to the increase in cosmic-ray intensity that goes with an increase of some 400 feet in altitude, a very small fraction of the difference in cosmic radiation intensity between Denver and sea level. Therefore, at the same time that we consider the possible effects of strontium 90 from such concentrations, we may deduce from our everyday ordinary experience limits on the effects to be expected. None of the evidence on the occurrence of bone cancer or leukemia as a function of altitude has given us any reason to believe that the present tolerance limits are in any way in error. The present body burdens in new bones are small compared to these limits.

Separate from the strontium 90 effects are the effects of general gamma radiation, the radiation that is received mainly from outside the human body and which comes mainly from the very young fission products in the local fallout area, but which can come in smallest part from radiocesium accumulating on the ground in the case of the stratospheric fallout or, more importantly, from the shorter-lived fission products deposited by the tropospheric fallout. Of course, weapons tests are so conducted as to avoid exposures to local fallout; hence our present discussion of the effects of weapons will be restricted to the much smaller gamma-ray doses from the off-site tropospheric and stratospheric types of fallout. In time of war, of course, it would be the local fallout which would be of more direct concern, next to blast and thermal effects, and it is to this aspect of fallout that Federal Civil Defense Administration addresses itself in the main. In regard to nuclear tests, we have to study the effects on human genetics and the possible effects of such doses of

radiation on health. Let us again apply the criterion of normal human experience to this. Measurements have shown that the general average intensity of fallout gamma rays from tests is from 1 to 5 milliroentgens per year. Now the general magnitude of the effects to be expected from this can be compared with the natural radiation intensity. We find, as mentioned earlier, that such things as living in a brick house instead of a wooden house can produce as much as 25–50 milliroentgens extra dosage per year, that there are certain areas in the world where the average dose in this country of 150 milliroentgens per year is exceeded by tenfold, that people living on granitic rock as compared with those living on sedimentary rock receive about 70 milliroentgens per year more dosage due to the higher content of uranium and thorium in these rocks, and that people living at higher altitudes have a higher natural cosmic-ray dosage. Also, of course, we know that medical uses of X-rays can be considerably larger than any of these fallout dosages.

We do have experience and valid evidence that the somatic effects other than cancer and leukemia, that is, the effects of radiation on ordinary human health, require dosages which are very much larger—of the order of 25–50 roentgen units—in order to be observed as changes in the blood, and 100–200 roentgens for injury symptoms, whereas the dosages we are speaking of, from test fallout, are about one hundred thousand fold smaller.

As for genetic effects, these are extremely difficult to evaluate, since so little is known about human genetics. But, judging from experience with plants, insects, animals, and lower organisms, there is every reason to expect some genetic effects of radiation. The question is how much radiation is required for a given level of effect. There are a certain number of mutations in every new human generation. Are these largely induced by natural radiation, or are they mainly of chemical, or rather biochemical, origin, or both? From a chemical point of view, it seems likely that not all the spontaneous mutations in the human or any other species are caused by radiation effects, because it seems likely that radiation acts in inducing mutations mainly via molecules which it generates in the human cell, and that the mutations are caused by these chemicals and therefore in a sense are chemical in nature. Now, if this is so, and the radiation-induced mutations are nearly always caused by chemicals which are produced in the first instance by radiation, then chemicals which are not themselves produced by radiation, but have other origins, can cause mutations; thus it seems likely that a major part of the natural or spontaneous mutations in any species is not radiation-induced. This point is an important one to settle, for the reason that we have to compare the effects of fallout radiation with the fraction of the natural spontaneous mutations due to the radiation to which we are normally subjected. In other words, if the normal mutations are all due to radiation, then the effects of the additional radiation from general test fallout, or from other sources of radiation such as atomic power or the medical uses of isotopes and X-rays, will be larger. It seems likely, and many genetic authorities agree on genetic grounds with this conclusion, that a major portion of the spontaneous mutations of the human species is due not to radiation but to other causes. Therefore, a fraction of the spontaneous mutations in the human species is taken as being due to irradiation. Now, what this fraction is, it is difficult to say; but Professor H. J. Muller has estimated that this might be 10 per cent. Therefore, one estimates that the 150 milliroentgens per year from

natural radiation now causes about 10 per cent of the spontaneous mutations and, therefore, that the test fallout, if continued indefinitely, will, at the present level of about 1–5 milliroentgens per year, cause an increase in the natural spontaneous mutation rate of something like one-fiftieth of 10 per cent, or 0.2 per cent of the spontaneous mutations. In the extreme, if it should prove that all the spontaneous mutation rate is radiation-induced despite the chemical arguments, the effect would be ten times as great, or 2 per cent. Dr. Gordon Dunning, of the Division of Biology and Medicine of the Atomic Energy Commission, estimated 1.4 per cent in 1955, based on the assumption that the annual fallout was equal to the highest amount experienced heretofore in any one year, and that 10 per cent of spontaneous mutations were due to natural radiation (*Scientific Monthly*, **81**, 265, December, 1955). This effect is one which is comparable to moving to a slightly different locality and is much less serious than changing from one house to another or doing any of a dozen things. The only important point is that genetic effects appear only if large numbers of people are subjected to them. Therefore, we would expect that the effects of large populations changing their environment—for example, living at a higher altitude or living in a region of naturally higher radioactivity—should cause genetic effects if test fallout does so. An examination of vital records should be made to test for such effects, and the Atomic Energy Commission is doing so as best it can. The United Nations Scientific Committee on the Effects of Atomic Radiation has been comparing the data on natural background dosages, and it is hoped that this study will be continued and that the search will be made for observable effects of variations in the natural background dosage, for it is certain that any effects due to gamma rays from fallout must be already present in much larger measure due to the natural dosage.

II. VARIATION IN INDIVIDUAL STRONTIUM 90 BURDENS

What is the likelihood that even if the average strontium 90 content is well within tolerance limits, a few individuals should exceed tolerance limits? Let us consider first the case which will ultimately hold, the situation of complete equilibrium with the environment in so far as the strontium 90 burden is concerned. The only way we can make judgments about the expected individual variations from the mean concentration is by direct experiment on human-body composition, not only for strontium 90 but for other analogous constituents. Most of the recent data on the strontium 90 body burden are from odd bits of bone removed during surgical operations, but fortunately we have actual data for the strontium 90 content of the entire bodies of some several dozen stillborn children[1] in the city of Chicago in the year 1953. A strenuous effort is now being made in Project Sunshine to continue this series and also to check the human-bone data by analyses of complete skeletons. We present the distribution of the strontium 90 data for the stillborn children in Figure 1. Data for the occurrence of ordinary nonradioactive strontium in human bones also have been published.[2] These obviously refer to the full steady-state condition and are obviously at least as nearly in equilibrium with the environment as the fallout radioactive strontium ever will be. These data are presented in Figure 2. The occurrence of radium in the human body also has been used, since it is chemically similar to both calcium and strontium and therefore is a bone-seeker, and because it is obviously also in steady-

state equilibrium. The data used were those of Palmer and Queen[3] (Fig. 3) And, finally, we use the recent data on occurrence of normal potassium in human bodies as determined by Anderson and Langham[4] at the Los Alamos Scientific Laboratory, presented in Figure 4. All these data show a normal frequency distribution as indicated by the theoretical curves. The respective widths of the curves (standard deviations) are 36 per cent for radiostrontium, 40 per cent for normal strontium, 40 per cent for radium, and 18 per cent for natural radiopotassium. It is completely clear from these data that the distributions agree with one another in general shape and that the magnitude of the distribution of the strontium 90 contents of the Chicago stillborn babies was not in any way anomalous. Therefore, we shall take the distribution curve for radiostrontium to be the same as for the normal strontium data. The occurrence of nonradioactive normal ordinary strontium in the bones should certainly tell us what the equilibrium distribution will be for radioactive strontium, and from it we should be able to learn the points about distribution which we cannot yet learn in any detail from the radioactive strontium itself. Turekian and Kulp noted in their study of normal strontium in human bone that in a given region the deviation from the average was about 34 per cent of the average, that is, for human bone from the regions Colorado, Texas, Cologne, Bonn, Venezuela, Chile, Vancouver, China, and India. In each instance the ratio of the standard deviation from the mean itself was taken and the average calculated, to obtain 34 per cent. Therefore, we take 34 per cent as the expected standard deviation from the mean *for a given locality* for the eventual strontium 90 equilibrium burden in human bones.

With this result we can, assuming a normal error-curve shape of the distribution of probabilities, answer the immediate question: What is the probability of an individual exceeding the tolerance even though the mean does not? On the basis of this analysis, we find that at steady state and in equilibrium the variation from the mean will constitute an error curve with a shape corresponding to the standard deviation, being one-third of the mean. Therefore, at steady state among people living in a given locality, only one person in about 700 will have more than twice the average strontium 90 burden, and the chances of anyone having as much as three times the normal burden will be about one in twenty million.

Now, what about the nonequilibrium distribution, when the strontium 90 is finding its way into the biological system? Obviously, the burden will be much lower here, but the deviation from the mean will probably be much higher percentagewise, particularly in adults, where most of the bone has been deposited before strontium 90 was produced. The present strontium 90 content of adults depends very much on the growth rate and the metabolic activity of the various bones in the given individual's body which happens to be sampled. However, the specific concentration of the strontium 90 deposited will not exceed that in new bone developed entirely in the present biological environment, i.e., the local concentration in adult bone will not exceed that for the whole bone in young children, whose total bodies are composed of the mixture of strontium 90 and calcium which now is present in food. Since the present child-adult ratio is about 4 to 1 for average total strontium 90 content, the factor of concentration in adults' active bone regions may be as much as fourfold greater than the whole-body average. Thus the apparent spread for random bone samples taken from adults should be very

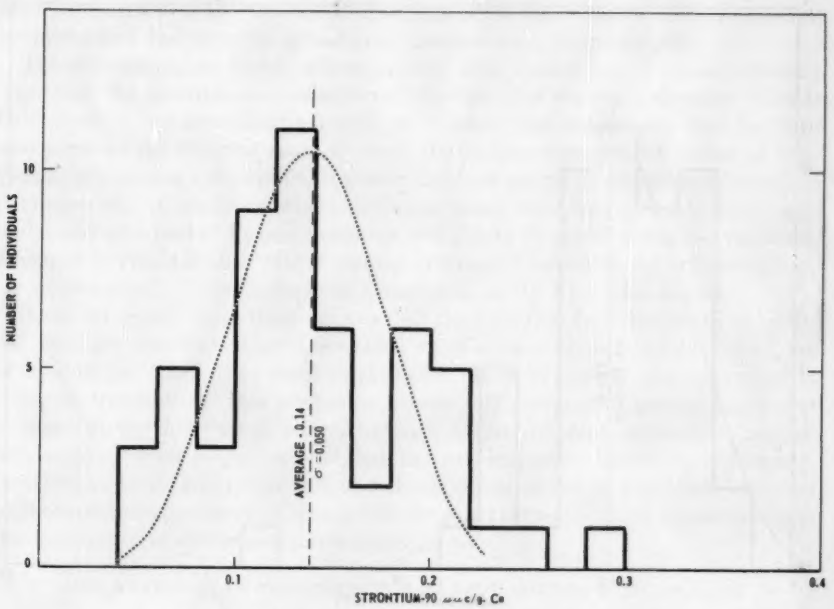

FIG. 1.—Radio strontium (Chicago, 1953; Stillborns)

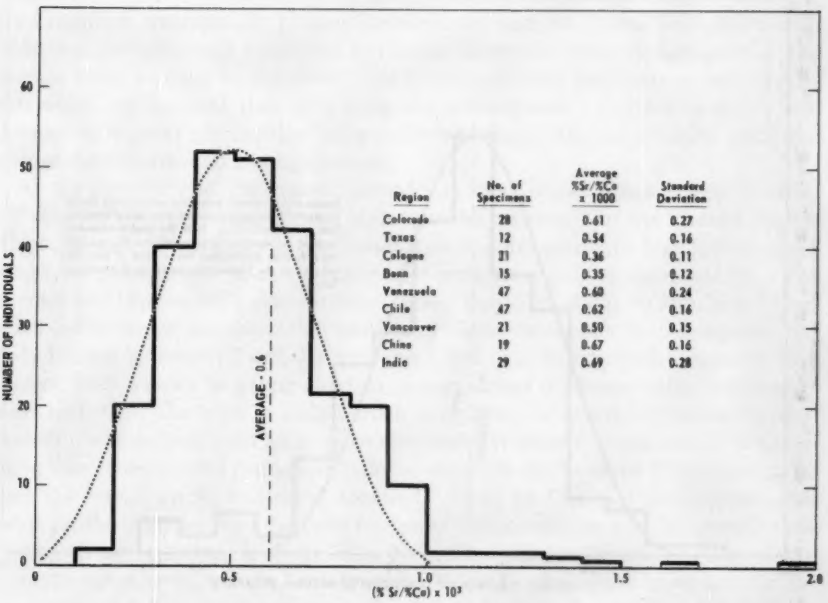

FIG. 2.—Normal strontium (Turekian and Kulp)

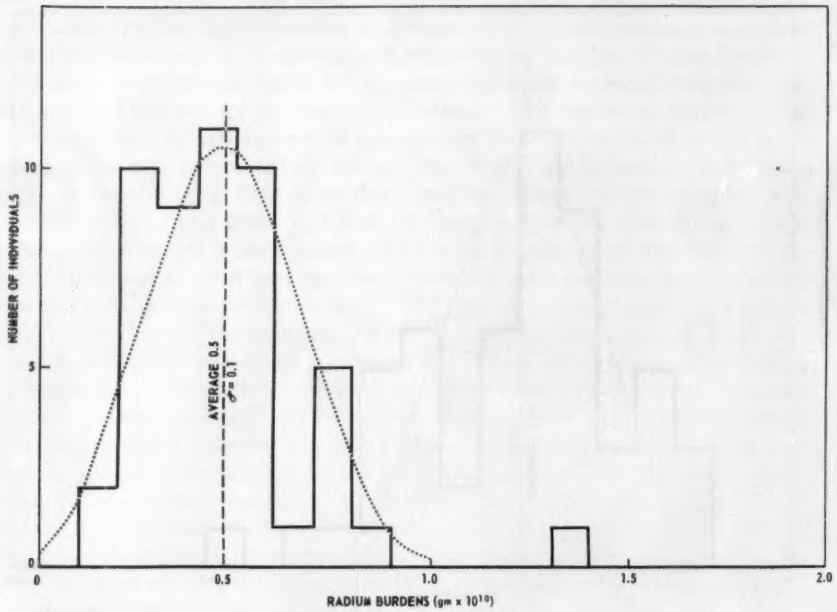

FIG. 3.—Radium (Palmer and Queen, MW-31242) (no correlation with drinking water)

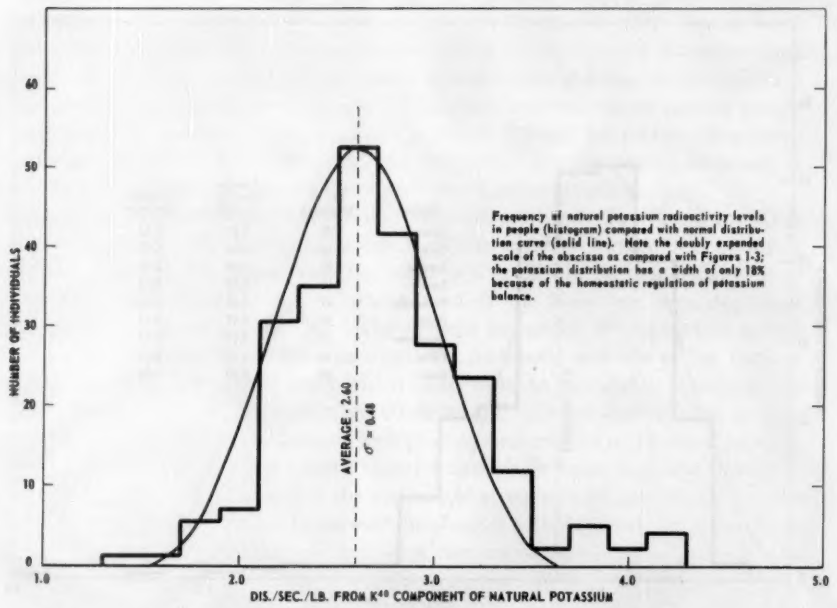
NUMBER OF INDIVIDUALS
AVERAGE : 2.60
σ = 0.48
Frequency of natural potassium radioactivity levels in people (histogram) compared with normal distribution curve (solid line). Note the doubly expanded scale of the abscissa as compared with Figures 1-3; the potassium distribution has a width of only 18% because of the homeostatic regulation of potassium balance.
0
10
20
30
40
50
60
1.0
2.0
3.0
4.0
5.0
DIS./SEC./LB. FROM K⁴⁰ COMPONENT OF NATURAL POTASSIUM

FIG. 4.—Potassium (Anderson and Langham, unpublished) (age group, 20–39 years)

large compared with the true equilibrium spread for these reasons. As equilibrium is approached, however, the spread must decrease very, very markedly.

The data on human bones indicate a very wide scatter, but it seems extremely clear that the variation is a reflection of the fact that the main skeleton of adult individuals is not in equilibrium with the present food supply and that the variations reflect the different rates at which the various bones in the bodies of individuals are coming into equilibrium with the food supply in the general biological environment. A study of whole skeletons taken from one given locality, now under way as a part of Project Sunshine, will clarify the point about the variations among individuals in their rate of coming into equilibrium with the general biological environment. This study is being carried on in Dr. Kulp's laboratory.

It should appear from these studies that the variation from the mean of adults will be larger than the factor of one-third which apparently is normal for the types of equilibrium distribution considered above. It is, of course, very important to establish the truth of this prediction clearly. However, the general agreement in shape of the distribution curves for such widely different materials as normal potassium in whole bodies, radium, and normal elementary strontium in fragmentary human bone, and actual fallout radioactive strontium in the whole bodies of stillborn children give us good reason to believe that there is nothing extraordinary in the distribution of radiostrontium in human bone.

III. VARIATION OF THE STRONTIUM 90 BODY BURDEN WITH LOCALITY

Most important of the causes of variation of the strontium 90 content of individuals with locality is, of course, the amount of fallout in a given region. The general rules about the intensity of fallout have been described above. For air-fired megaton weapons our present indication is that the fallout is almost world-wide, and for reasons of simplicity and in the absence of better information at the present time, we work on the model that this is a uniform distribution, over the entire world, of material that falls from the stratosphere. Further evidence and data on this point are rapidly being collected which will undoubtedly settle the stratospheric horizontal mixing question.

At the present time, the general latitudes in the Northern Hemisphere between 10° and 60° N. have the highest strontium 90 content. In the United States, which, because of proximity to the Nevada test site, has unusually high fallout, there are at the present time about 25 millicuries per square mile of strontium 90. For average soil this means a concentration in the topsoil of about 50 Sunshine Units. With the factors of discrimination mentioned above, this means that an equilibrium body burden between 1.7 and 3.9 Sunshine Units is to be expected. Actually, the present body burden in young children indicates that the lower value is probably more realistic. The present body burden in children—about 0.5 Sunshine Units—probably was derived from an average strontium 90 content in the topsoil of something like 15 millicuries per square mile, or about 25–30 Sunshine Units during the time the strontium 90 was being acquired. Thus we find that the experimental value for the ratio between the body burden of young children and the average concentration in the topsoil is about 50 to 1—rather closer to the higher range of the laboratory results than to the lowest range. Table 2 contains the latest data for the total strontium 90 fallout as measured in United States soils, and Figure 5 displays

these data graphically. The northern part of the United States has about 20–30 millicuries of strontium 90 per square mile, the southern states somewhat less. The low figure of 7 millicuries per square mile for Grand Junction, Colorado, is probably due to local climatic and sample-site conditions.

TABLE 2*

HEALTH AND SAFETY LABORATORY 1956 SURVEY OF U.S. SOILS FOR STRONTIUM 90 SAMPLES TAKEN BETWEEN OCTOBER 8 AND 13, 1956

SAMPLING SITE	DEPTH (INCHES)	DISINTEGRATIONS/ MIN/GM SOIL	MC/MI²	MC/MI² Av.	MC/MI² Total
Albuquerque, N.M.	0–2	0.078 ± 0.001	7.5 ± 0.1	7.3	
		0.075 ± 0.001	7.2 ± 0.1		
	2–10½	0.008 ± 0.002	4.4 ± 0.9	3.4	11
		0.005 ± 0.002	2.4 ± 0.8		
Atlanta, Ga.	0–2	0.35 ± 0.007	14 ± 0.3	15	
		0.42 ± 0.009	16 ± 0.4		
	2–6	0.018 ± 0.004	2.8 ± 0.6	3.0	18
		0.021 ± 0.003	3.3 ± 0.5		
Binghamton, N.Y.	0–2	0.32 ± 0.007	17 ± 0.4	18	
		0.35 ± 0.007	18 ± 0.4		
	2–6	0.019 ± 0.003	4.4 ± 0.8	5.0	23
		0.024 ± 0.005	5.6 ± 1.1		
Boise, Idaho	0–2	0.23 ± 0.006	20 ± 0.6	22	
		0.26 ± 0.006	23 ± 0.6		
	2–6	0.012 ± 0.002	3.1 ± 0.6	3.5	26
		0.015 ± 0.002	4.0 ± 0.6		
Des Moines, Iowa	0–2	0.31 ± 0.007	23 ± 0.5	23	
		0.31 ± 0.007	23 ± 0.5		
	2–6	0.028 ± 0.002	7.6 ± 0.7	7.1	30
		0.024 ± 0.003	6.6 ± 0.7		
Detroit, Mich.	0–2	0.26 ± 0.006	20 ± 0.5	20	
		0.27 ± 0.006	20 ± 0.5		
	2–6	0.038 ± 0.003	7.3 ± 0.5	7.8	28
		0.044 ± 0.003	8.4 ± 0.6		
Grand Junction, Colo.	0–2	0.10 ± 0.001	7.8 ± 0.1	7.0	
		0.091 ± 0.001	7.1 ± 0.1		
		0.11 ± 0.019	8.2 ± 1.4		
		0.070 ± 0.013	5.1 ± 1.0		
	2–10½	≤0.002	≤0.45	≤0.48	7
		≤0.002	≤0.51		
Jacksonville, Fla.	0–2	0.11 ± 0.009	7.3 ± 0.6	7.3	
	2–6	0.013 ± 0.004	2.7 ± 0.9	3.4	11
		0.020 ± 0.005	4.0 ± 1.0		
Los Angeles, Calif.	0–2	0.12 ± 0.008	6.9 ± 0.5	7.5	
		0.14 ± 0.009	8.0 ± 0.5		
	2–7	0.009 ± 0.002	3.3 ± 0.9	2.8	10
		0.006 ± 0.002	2.2 ± 0.7		
Memphis, Tenn.	0–2	0.27 ± 0.006	15 ± 0.4	15	
		0.26 ± 0.006	15 ± 0.4		
	2–6	0.028 ± 0.003	6.5 ± 0.7	6.6	22
		0.029 ± 0.003	6.6 ± 0.7		
New Orleans, La.	0–2	0.24 ± 0.006	8.8 ± 0.2	8.6	
		0.22 ± 0.006	8.3 ± 0.2		
	2–6	0.009 ± 0.002	3.3 ± 0.9	2.8	11
		0.006 ± 0.002	2.2 ± 0.7		
New York, N.Y.	0–2	0.21 ± 0.006	10 ± 0.3	12	
		0.29 ± 0.007	14 ± 0.3		
	2–6	0.072 ± 0.004	14 ± 0.8	14	26
		0.068 ± 0.004	14 ± 0.8		
Philadelphia, Pa.	0–2	0.17 ± 0.005	12 ± 0.4	12	
		0.16 ± 0.005	11 ± 0.4		
	2–6	0.029 ± 0.003	7.3 ± 0.8	6.8	19
		0.026 ± 0.003	6.4 ± 0.7		
Rapid City S.D.	0–2	0.29 ± 0.006	20 ± 0.4	22	
		0.34 ± 0.006	23 ± 0.4		
	2–6	0.053 ± 0.004	12 ± 1.0	11	33
		0.045 ± 0.003	10 ± 0.7		

TABLE 2*—(*continued*)

Sampling Site	Depth (Inches)	Disintegrations/ Min/Gm Soil	Mc/Mi²	Mc/Mi² Av.	Mc/Mi² Total
Rochester, N.Y.	0–2	0.22 ± 0.006	16 ± 0.4	16	
	2–6	0.013 ± 0.002	2.5 ± 0.4	2.5	19
		0.013 ± 0.002	2.5 ± 0.4		
Salt Lake City, Utah	0–2	0.32 ± 0.007	22 ± 0.5	22	
		0.33 ± 0.007	23 ± 0.5		
		0.31 ± 0.007	22 ± 0.5		
	2–8	0.016 ± 0.002	5.7 ± 0.7	5.8	28
		0.016 ± 0.002	5.9 ± 0.8		
Seattle, Wash.	0–2	0.46 ± 0.011	17 ± 0.4	17	
		0.44 ± 0.010	16 ± 0.4		
	2–6	0.051 ± 0.007	9.4 ± 1.2	9.5	27
		0.052 ± 0.004	9.6 ± 0.7		

* Strontium extracted with 6 *N* HCl at room temperature. Replicates represent individual soil aliquots taken after sampling and air drying. Each error term represents one standard deviation due to counting error.

The differential rates at which the fallout has been occurring probably are best measured by the so-called "pot collection" method. A bucket with vertical walls of appreciable height is placed out in the open and allowed to collect the total fallout for a given period, including the rain, snow, dust, etc. The bucket is left out whether it has rained or not and covers the total fallout for a given period. Figures 6 and 7 give the curves so obtained for the New York and Pittsburgh

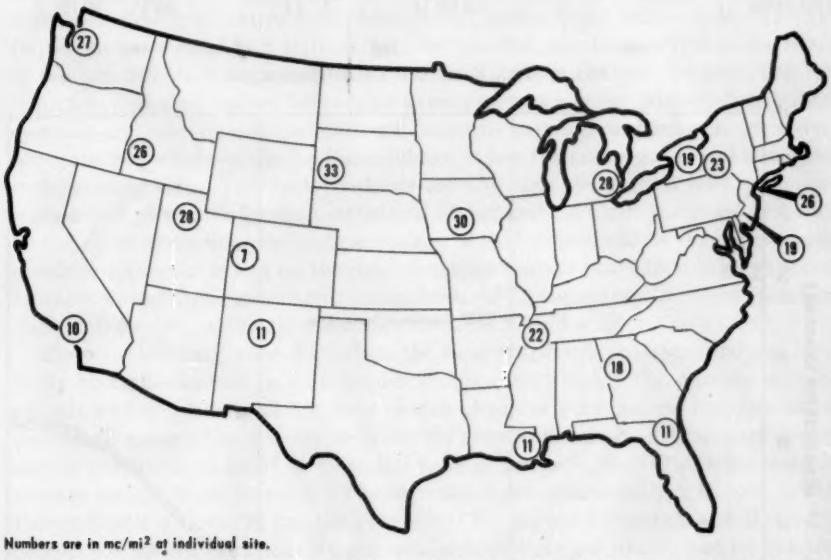

FIG. 5.—Strontium 90 in United States soil (HASL, October 8, 1956) (HCl extraction method).

areas, together with the estimated errors of measurement. It is interesting to note the changes in slope and to correlate them with the occurrence of test activities and the relatively short-lived tropospheric fallout. The minimum slopes which appear during quiet periods when no one is testing are the stratospheric fallout of which we have spoken, and these slopes, when we have enough pots operating all over the world, will, when taken together with the results of the measurements

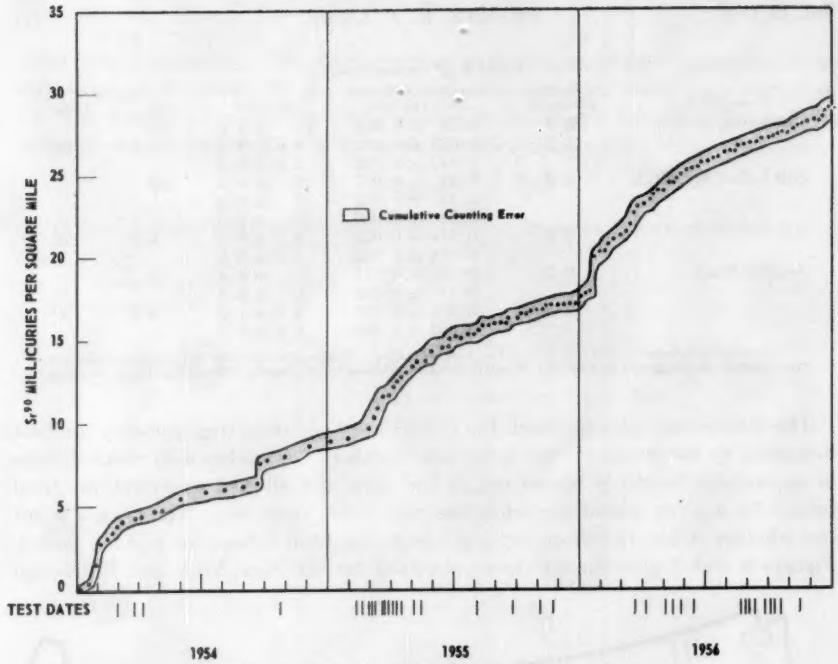

FIG. 6.—Rain data, New York collection pot

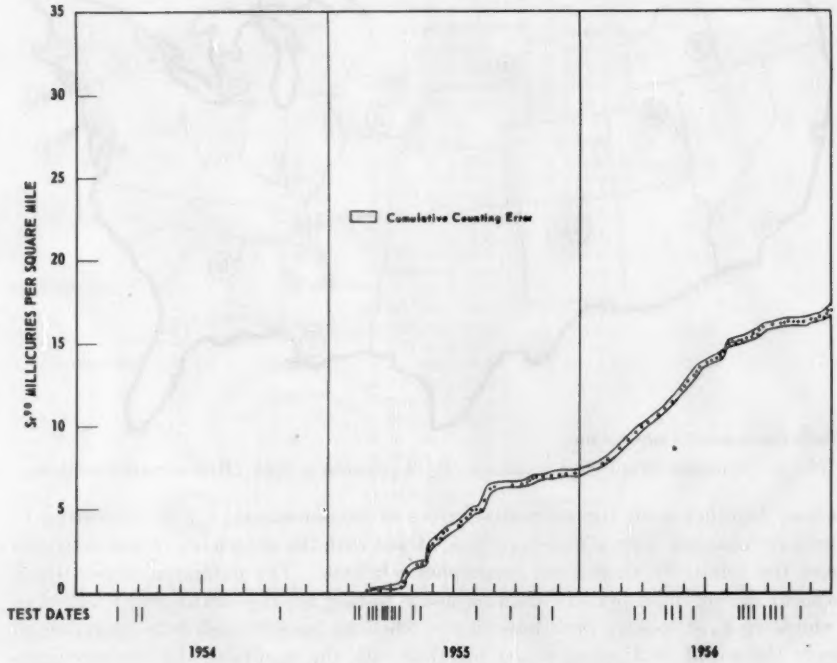

FIG. 7.—Rain data, Pittsburgh collection pot

of the amounts of radiostrontium and radiocesium in the stratosphere, give an accurate value for the stratospheric residence time and settle the mixing question.

In addition to the intensity of fallout, the question of the fraction of the radiostrontium, and, for tropospheric fallout, the radioiodine of 8-day half-life, that is in assimilable form is an important one. So far, most fallout strontium appears to be completely water-soluble and therefore most assimilable, though continued tests on this point should be made. Direct leaf pickup of course promotes assimilation of the strontium, because the plant differentiation against strontium when it assimilates it from soil thus is avoided. Another factor is, of course, the concentration of available calcium in the soil. By "available" calcium we mean calcium which is available to plants and not the total calcium in the soil. It is known that soils which are high in available calcium produce plants of lower radioactive strontium content; that is, the radioactive-strontium-to-calcium ratio in the plant is lower as a direct consequence of the lower concentration of radiostrontium in the available soil calcium. In addition, as mentioned previously, plants tend to prefer calcium to strontium with a discrimination factor of about 1.4. Sheep which grow in certain areas of Wales have shown concentrations in their bones approaching 150 Sunshine Units, while sheep and cattle growing in the United States have hardly ever exceeded one-fifth of this. The Welsh soil in certain areas is very low in calcium and, as a result, grows grasses of high radiostrontium content. Of course, it is clear that fertilization with calcium will immediately relieve this difficulty; but in the absence of such fertilization, the question is: How serious is the effect of calcium deficiency in promoting strontium 90 pickup through the food chain?

As was remarked earlier, there is an averaging which occurs in food distribution systems, and calcium-deficient soils are naturally rather poor producers; as a consequence, the weight of the food so produced is less than for a good, well-fertilized, well-balanced soil. This factor reduces the flow into the general food system of material of exceptionally high strontium 90 content. It will therefore probably be sufficient to consider the radiostrontium of milk, since milk is the main source of calcium, in order to test for the radiostrontium content of the food in given areas. Direct measurements have shown that a factor of 5 encompasses the total variation due to all factors, including calcium deficiencies in acid soils.

The general intake must depend on the food distribution pattern, and the relatively small fluctuation in milk contents must reflect this. The number of individuals who rely totally on the food output of soil of very low calcium content is very small indeed, but it must be true that these individuals, if they grew up on such a provincial, isolated farm, would have as much as 10–50 times the normal average strontium 90 content. The normal calcium concentration in soils in the United States is about 20 gm. per square foot for the top 2.5 inches, and about the poorest soil known has about 0.4 gm. available calcium per square foot for the top 2.5 inches—a deficiency factor of 50.

It is clear from a detailed examination made by the author for people living in calcium-deficient areas with normal food distribution patterns that a factor of 5 is about as large an effect as can be expected from a fifty-fold deficiency of calcium in the soil. The food from outside the calcium-deficient area reduces by a factor of about 10 the increase in strontium 90 pickup rate which would be expected from the calcium deficiency in the soil if people lived entirely off the soil for their whole growing period of 20 years or so.

The food of lowest strontium 90 content is fish flesh, because of the great dilution the fallout receives by the hundred meters of sea water above the thermocline, which rapidly mix with the fallout within a few hours or days. This means that the specific concentration of radioactive strontium, or any other fallout constituent in sea water, is relatively very much lower than it would be in soil. For example, 100 meters of sea water has 370 gm. of dissolved calcium per square foot, as compared with the average of 20 gm. per square foot for the top 2.5 inches of soil which absorbs and holds the fallout radiostrontium. Therefore, in principle, sea food and fish are lowest among foods in content of radiostrontium fallout.

IV. EFFECTS OF CONTINUED TESTING AND GENERAL CONCLUSIONS

In summary, then, we see that the present body burden of strontium 90 from atomic weapons tests in the United States corresponds to the radiation dosage to the bones which would result from a few hundred feet increase in altitude, and the present vital statistics show no observable effect on the occurrence of bone cancer or leukemia of much larger changes in altitude. The tolerance figure of 100 Sunshine Units, or 0.1 microcurie for an average individual, or 100 micromicrocuries per gram of body calcium, that is recommended now is about two hundred times the present level for new bone in the United States, and it will not be exceeded by fallout from weapons tests in any foreseeable circumstances.

The distribution of strontium 90 burdens among individuals *for a given locality* will be a normal error curve with a standard deviation of about one-third of the average concentration. This means that about one individual in 300 will have more than twice the normal average value for a given locality, and that about one in several million will have three times the average value.

The effect of locality is more important, however, particularly in the effect of calcium deficiency in the soils. Careful consideration of this question indicates that there will be a very few individuals who show a strontium 90 content which is strictly inversely proportional to the available calcium concentration of the soil in their region. This is due to the fact that food distribution systems automatically average over a wide area, and people assimilate their calcium slowly. Most people drink milk and eat cheese and other calcium-bearing foods from a rather wide area, and this effect reduces by an estimated factor of 10 the potential effect of calcium deficiency in the local soils.

On the basis of laboratory experiments, the human-body concentration of strontium 90 at equilibrium will be between 13 and 30 times less than that in the topsoil. The present data indicate that the higher figure is closer to the truth, and so we will be conservative in taking the figure of 20 for this ratio. Therefore, the present burden of 50 Sunshine Units in the topsoil of the United States may eventually lead to as much as 2.5 Sunshine Units in human bones but more likely will lead to about 1.7.

Of course, as testing continues and more fallout occurs, the levels will rise. The strontium 90 that still resides in the stratosphere at the present will fall out, according to our expectations, at a rate which just about compensates for the decay of the material already deposited, so that no great additional increase from this source is to be expected from weapons fired in the past. If the testing should continue at about the same rate it has averaged over the last five years, then we should.

at equilibrium, after an infinite time, approach a level of 8 times the present rate, since the average life of strontium 90 is 40 years. This assumes that future testing will be conducted so as to give in each future five-year period the same as the last five have. And so we would expect in the United States at that time an average human strontium 90 concentration of 20 Sunshine Units, with the conservative factor of 20 between the topsoil concentration and the concentration in human bone, or 5 Sunshine Units if the factor of 80 is used. In other words, in the United States something between 5 and 20 Sunshine Units would be the equilibrium concentration of human bones if testing continued indefinitely at the average rate of the past five years. This level would be approached after only a few decades. After 28 years the level would be half this equilibrium value, and after another 28 years—56 years total—from an arbitrary beginning which we have set as 1952, we would expect in the year 2008 three-fourths of the equilibrium figures. So somewhere between 4 and 15 Sunshine Units of strontium 90 in human bones in the United States might result from the present type of testing being continued for the next 50 years.

In those particular areas in the world where the soil is low in calcium, this level might go fivefold higher. At the present rate of testing, we might indeed approach the figure of 100 Sunshine Units, the tolerance limit for large populations, at the beginning of the twenty-first century for these certain limited regions in the world. The observed conditions in these regions could be relieved, however, by fertilization of the soil with calcium, using either calcium nitrate or lime, as appropriate from other considerations.

Project Sunshine continues to study the problems of world-wide fallout—the stratospheric inventory of radiostrontium and radiocesium, the occurrence of these isotopes in the soils and water and the biosphere all over the earth, the biological effects at certain levels of contamination with strontium 90, and to a lesser degree with cesium 137, and the possible genetic effects of the low gamma-ray dosages associated with world-wide fallout from atomic tests. All these are studied not only with the object in mind of devising methods of protection against atomic warfare, but also with the thought of possible application in the remote event of industrial accidents which may happen in connection with certain of the peaceful uses of atomic energy, particularly atomic power. Certainly an understanding of the basic principles of world-wide fallout is applicable to the control and safe handling of isotopes. All of this is done in collaboration with the United Nations Scientific Committee on the Effects of Atomic Radiation, and it is to be hoped that, as the data appear, all the countries in the world will join together in this international effort to understand better the effects of the great new fact in life, the nuclear atom.

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*THE LOCALIZATION OF BASIC PROTEINS IN THE NUCLEI OF LARVAL DROSOPHILA SALIVARY GLANDS**

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The identification and specific localization of chemical constituents within the cell nucleus have lagged considerably behind the qualitative and quantitative characterization of these classes of substances, with the possible exception of one component, the deoxyribonucleic acid (DNA). In addition to the latter, the nucleus contains ribonucleic acid[1, 2] (RNA), histone or basic protamine[3] (BP), and at least one other type of protein, the chromosomin of Stedman and Stedman,[4] the tryptophane-containing protein (Tr.Pr.) of Mirsky and Pollister,[5] or the non-histone protein fraction which Hamer[6] has subjected to comparative amino acid analysis. The localization of these substances in the live nucleus is dependent almost wholly on inference from a variety of analyses and observations made on fixed preparations. The much disputed and maligned Feulgen reaction points to the DNA as a major constituent of the chromosomes. Studies with ultraviolet absorption spectroscopy,[1] enzymatic attack, and pyronin staining[2] indicate that the RNA of the nucleus is predominantly nucleolar. The distribution of the BP seems to be much less precisely delineated. For example, Caspersson[7] has reported its presence in the chromosomes and nucleolus of *Drosophila* salivary gland nuclei from his ultraviolet absorption studies. Vincent,[8] from his chemical studies of the isolated nucleoli of starfish oöcytes and of maize, could find no basic protein. Bloch and Godman[9] have reported a proportional quantitative relationship between DNA and histone synthesis per rat fibroblast or liver cell. They have also shown cytochemically that, in a general manner, DNA and histone have the same nuclear distribution.

In the present study the Feulgen reaction for DNA localization, combined with the fast-green-staining method of Alfert and Geschwind[10] for basic protein, has been employed with *Drosophila* salivary gland material to determine more precisely the localizations of BP with respect to DNA.

The method employed is essentially that of Bloch and Godman:[9] salivary glands of *D. virilis* fixed with trichloroacetic acid (TCA) were partially crushed under a cover slip; these preparations provided some whole nuclei. After the slide was frozen on a block of "dry ice," the cover slip was flicked off and the preparation stained directly by a modified Feulgen technique following 24 hours' lipid extraction in 95 per cent alcohol. Other salivary squashes were prepared similarly, using the conventional acetic acid fixative that permits rupture of the nuclei, yielding fully extended chromosomes. After a Feulgen treatment in which TCA was substituted for HCl, photographs were taken of a number of chromosomes or of whole nuclei; the position of each was recorded on a calibrated mechanical stage. The preparations were then re-exposed, and, after TCA extraction (15 minutes at 90° C.) to remove the DNA, were stained by the fast-green technique of Alfert and Geschwind. The recorded fields were then rephotographed.

The results are illustrated in the accompanying figures. In both the ruptured (Figs. 1–3) and unruptured nuclei (Fig. 4) there was a clear band-for-band staining correspondence between the Feulgen-treated chromosomes (Figs. 1*A*, 2*A*, and 3*A*) and their DNA-extracted fast-green-stained remnants (Figs. 1*B*, 2*B*, and 3*B*). Diffuse heterochromatin of the chromocenter stained after both treatments. The nucleolus was completely unstained by the Feulgen reaction, although its presence was frequently indicated by the Feulgen-positive reaction of the nucleolus-organizing region (Fig. 1*A*, *arrow*). After the fast-green treatment, the nucleolus, which often appeared vacuolated, and its organizing region were clearly evident (Fig. 1*B*). Their position invariably was neither against the nuclear membrane nor in a central position but rather near the chromocenter.

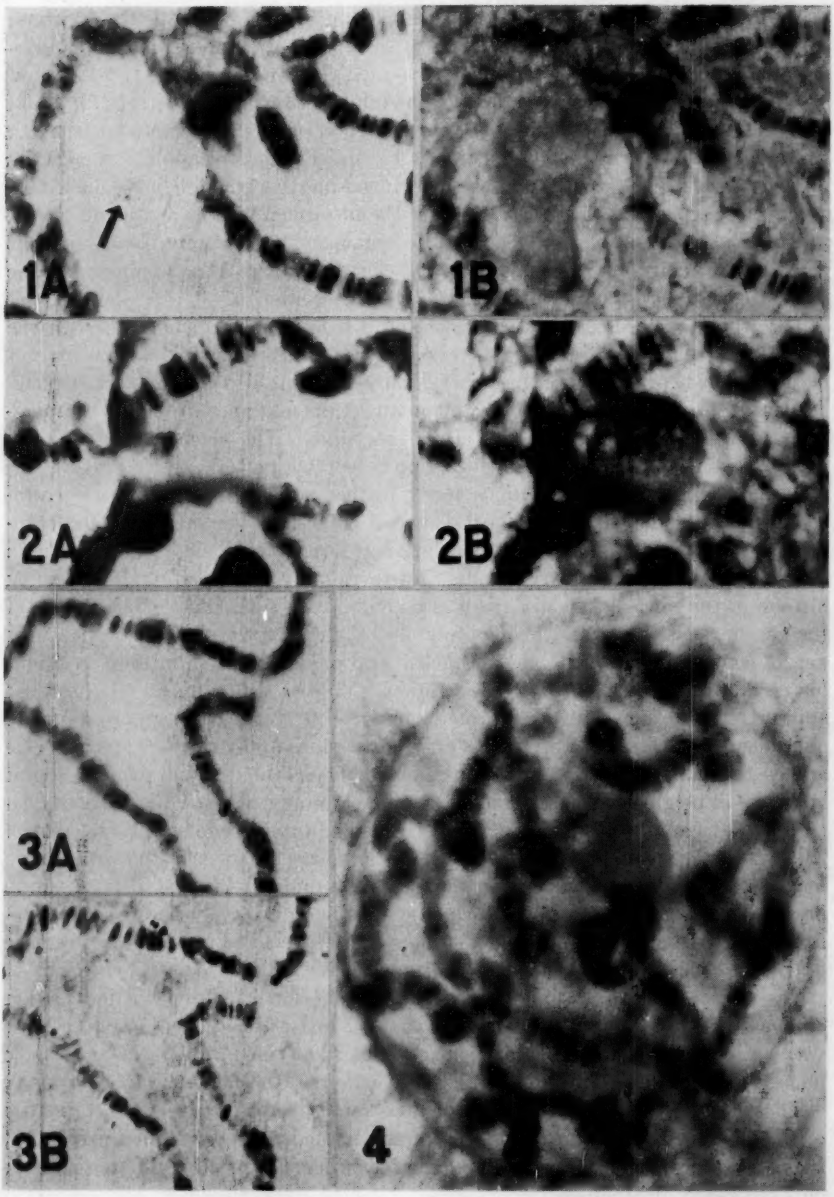
The whole nuclei (Fig. 4) clearly showed a heavier fast-green reaction in the region of the nuclear membrane; the exact location of this concentration could not be determined, primarily because the gland cytoplasm also presented a strongly positive fast-green reaction. In unbroken nuclei the materials of the interchromosomal space gave no reaction with either procedure. The placement of the ends of chromosomes in whole nuclei showed no detectable pattern.

A number of these observations merit further comment. The precise correspondence of histone and DNA in the banded portions of the salivary gland chromosomes confirms Caspersson's[7] conclusion drawn from ultraviolet absorption studies on *Drosophila* salivary glands. Furthermore, Caspersson reported finding histone in the nucleolus, a discovery also confirmed by our data. The failure of the chromosomal interband regions to stain by either cytochemical reaction does not, of course, prove the absence of nucleic acid or basic protein in these positions but certainly indicates a lower concentration. Vincent's[11] argument relating what appears to be an unusually high protein content (40–90 per cent) reported for the nucleolus with its staining properties does not seem to apply to these studies. He argues that with such concentrations the abundance of diamino acids per unit volume will be greatly increased and that therefore any reaction based on the detection of the amino groups (e.g., Sakaguchi) will always be a relatively strong one. Alfert and Geschwind[10] indicated that the intensity of their fast-green-staining reaction seemed less dependent on the absolute number and type of basic group involved than on the over-all balance of acid to basic groups. The positive reaction of the nucleolus, then, in these studies is interpreted to indicate the presence of BP; the concentration would be a foolhardy guess.

Vacuolated nucleoli have been reported (see Vincent[11] for review) under other circumstances. It should be noted here that the fast-green-stained material shows vacuolation of the nucleolus particularly clearly in preparations of the whole nuclei. The vacuoles vary in size and in distribution throughout the nucleolus.

The similarity of staining reaction demonstrated by the diffuse heterochromatin of the chromocenter to that of the euchromatin lends support to the notion that these two types of chromatin are fundamentally alike chemically, differing perhaps only physically and temporally.

Since the interband regions must be accounted for chemically and since the remaining ill-defined protein fraction (chromosomin, Tr.Pr., acid-insoluble protein, residual protein, non-histone protein) remains unaccounted for in the nucleus, at least these portions of the chromosomes might by inference be thought to consist



FIGS. 1–4.—Photomicrographs of *Drosophila* salivary gland preparations stained first with the Fuelgen reaction (Figs. 1*A*, 2*A*, and 3*A*) and then, following DNA extraction, with fast green at pH 8 (Figs. 1*B*, 2*B*, and 3*B*). Figure 4 is of a whole nucleus prepared with fast green at pH 8 after DNA extraction. The arrow in Fig. 1*A* indicates the nucleolus-organizing region.

of this fraction. Such a scheme is not meant to imply limitation of this protein fraction to the interband region but rather should lend credence to a chromosome structure consisting of a "backbone" constructed of this protein along which the nucleoprotein complex is distributed finitely with spatial and chemical irregularity.

The large interchromosomal spaces seen in fixed intact nuclei would be predicted from the work of Buck and Boche[12] on living salivary glands. They observed simultaneous chromosome shrinkage and nuclear swelling in live material as it was fixed with acetic acid. The present material cannot under the circumstances provide evidence for or against the existence of "nuclear sap." One should be reminded, however, that Buck and Boche characterized the living salivary chromosome as "behaving chemically somewhat like a sponge, in that it is capable of taking in and giving out an astonishingly large proportion of its fluid content, without permanent alteration of its fundamental internal structure." If at fixation, with its concurrent shrinkage of the chromosomes, materials are "given out," then they must not be Feulgen-positive nor fast-green-positive; in other words, at fixation neither DNA nor histone is released to the interchromosomal space in quantities detectable by the cytochemical techniques employed.

The fast-green-positive reaction of the cytoplasm of these glands is especially puzzling. The Alfert and Geschwind technique has been applied by us to a wide variety of tissues, both embryonic and adult, but never with the strongly positive cytoplasmic reaction seen for salivary glands. It is possible, of course, that some wholly unrelated substance elaborated by these larval structures produces a positive reaction following the application of the fast-green technique (the results are identical whether or not the Feulgen reaction precedes the fast green). A modified Sakaguchi reaction[13] for the estimation of arginine showed the cytoplasm to be strongly positive, suggesting by inference that the positive reaction of the cytoplasm to the basic fast green was due to the presence of histone outside the nucleus.

Summary.—The successive treatment of *Drosophila* salivary gland nuclei with the Feulgen reaction for DNA and the fast-green technique of Alfert and Geschwind for basic protein has demonstrated an exact correspondence in position of these two chromosomal constituents. The nucleolus also appears to contain some basic protein.

We are especially indebted to Miss Mary Elizabeth House for preparing the majority of the tissues and for running the cytochemical reactions.

* This work was supported in part by research grant RG-4203 from the National Institutes of Health, Public Health Service.

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*A LABORATORY STUDY OF THE EFFECT OF REMOVAL OF NEWBORN ANIMALS FROM A POPULATION**

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If *Daphnia* are maintained in the laboratory under conditions of constant temperature and constant food ration, a characteristic pattern of population fluctuations is observed.[1-3] Population size at any temperature is determined exclusively by food increment.[3] Natural populations of Cladocera also seem limited by food supply.[4]

Removal of a constant percentage of the newborn animals from a *Daphnia pulex* population reduces the size of the residual population as a nonlinear function of the removal rate. The number of animals removed (i.e., the "yield," if we think of the *Daphnia* population as a fishery) is inversely proportional to population size. Total fluctuation, measured as the mean absolute difference between successive censuses of a single population, with the census made prior to the removal of the newborn, is independent of removal rate. In other words, if the number of animals in the population is counted every four days, the mean absolute difference between this number and the corresponding number determined four days earlier or later from the same population is independent of the number of newborn animals which are removed from this population on the completion of each census.

Fluctuations in residual population size are reduced in proportion to the removal rate of the newborn. That is, the mean absolute difference between the number of animals remaining in any particular population after removal of the newborn at any census and the number remaining in the same population after removal of the newborn at the subsequent or previous census is smaller, the higher the percentage removal of newborn.

Let V_T be the total variability in numbers, computed as the mean absolute difference between successive censuses of a single population, prior to the removal of newborn; V_P, the residual population variability, computed as the mean absolute difference between successive censuses of a single population, after the removal of the newborn; V_Y, the yield variability; P_F, the mean residual population size when F per cent of the newborn are removed at each census; P_0, the mean size of a population from which no young are removed; Y_F, the mean number of animals removed from a population in which the removal rate is F; F, the percentage of the newborn removed at each census. We can assign[5] the difference between V_T and V_P to the fluctuations due to the newborn animals which have been removed. That is,

$$V_T - V_P = V_Y. \tag{1}$$

The data of Slobodkin and Richman[5] indicate that

$$\frac{V_Y}{V_T} = \frac{F}{F + 2(1 - F)} \tag{2}$$

for all values of F.

Assuming the contribution of each animal to population fluctuation to be constant, equation (2) can be translated into the verbal statement that fluctuations in yield are proportional to removal rate of newborn, while total fluctuations are the sum of yield fluctuations and fluctuations in the residual populations. Residual population fluctuation is proportional to twice the percentage of newborn permitted to remain in the population.

The fact that population size is bounded would imply that any change in population size produced by an animal that will remain in the population must eventually be compensated for by an equal change of opposite sign. The animals which are removed, on the other hand, contribute only once to population fluctuation.

More recent data (which will be presented in detail elsewhere) indicate that both yield and population size are linearly and directly proportional to food supply over a wide range of food increment and removal rate. Some of these data will be discussed here in terms of yield and population size per unit food.

Since V_T is independent of removal rate, we can assume that

$$\frac{V_P}{V_T} = \frac{P_F}{P_0}. \tag{3}$$

Solving equation (2) for V_Y, rewriting V_P in equation (3) as $V_T - V_Y$, and substituting our solution from equation (2) for V_Y, we can write

$$\frac{P_F}{P_0} = \frac{V_T - V_T F/[F + 2(1 - F)]}{V_T}, \tag{4}$$

whence

$$P_F = P_0\left(1 - \frac{F}{F + 2(1 - F)}\right). \tag{5}$$

From equation (5) we can predict population size at any removal rate, given the size of the populations at 0 per cent removal. The agreement between calculated and observed values of P_F can be seen in Figure 1.

Note that the number of newborn animals is computed at each census as the difference between the sum of living and dead animals found at the current census and the number of live animals returned to the container at the previous census. There is reason to believe that some of the dead animals decompose completely in the interval between censuses, producing an overestimate of a priori removal rate. The populations shown in Figure 1 were maintained at a priori removal rates of 50 or 90 per cent. Utilizing the fact that occasionally, when no newborn animals are being produced, a certain number of animals cannot be accounted for, it was possible to estimate the percentage of the dead animals that decompose completely between censuses for each population, and adjust the estimate of actual removal rate accordingly. This has been done in Figure 1. Adjustment was not possible at 90 per cent removal, since newborn were always present.

The number of animals removed from the populations can be thought of as the "yield" from the populations. On the simplest assumption, yield from any self-regulating fishery should be equal to a conversion factor multiplied by the

difference in maintenance energy requirements between a fished and an unfished population, that is,

$$Y_F = K(P_0 - P_F), \tag{6}$$

where K is a conversion factor, in this case equal to 0.36.

The agreement between observed and calculated values of yield is shown in Figure 2. The aberrant points (†) are from two populations in which mean yield had not yet been stabilized. One of these was maintained at such a high food level that toxic effects of the algae may have been significant.

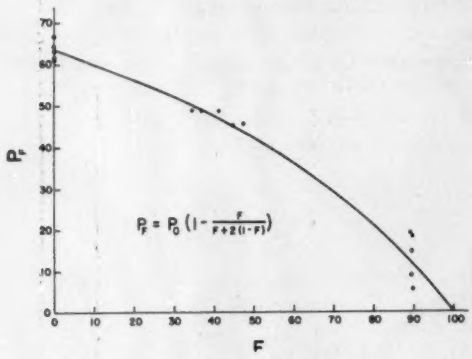

Fig. 1.

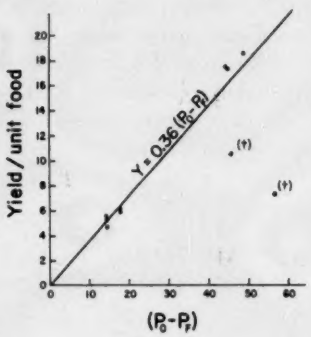

Fig. 2.

If the removal process is altered so that the number of animals removed remains a constant proportion of the newborn, *but adult animals are removed instead of young*, neither yield nor population size is directly proportional to food supply, the predictive equations are no longer valid, and it can be demonstrated that an appreciable portion of the food given to the populations is never actually consumed. The above equations therefore seem relevant only if the food is limiting.

Summary.—If a constant proportion of the newborn are removed from self-regulating populations of *Daphnia*, population size and yield are predictable from simple equations requiring only knowledge of the size of a normal population and one conversion constant. When adult animals are removed, destroying the simple food dependence of the population, these equations are no longer applicable.

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CONTENTS

Page

BIOCHEMISTRY.—ON THE IMPOSSIBILITY OF ALL OVERLAPPING TRIPLET CODES IN INFORMATION TRANSFER FROM NUCLEIC ACID TO PROTEINS . . . . . *By S. Brenner* 687

BIOCHEMISTRY.—INFECTION OF PROTOPLASTS BY DISRUPTED T2 VIRUS . *By John Spizizen* 694

BIOCHEMISTRY.—INCORPORATION OF RADIOACTIVE CARBON INTO A PTERIDINE OF A BLUE-GREEN ALGA . . . . . *By C. Van Baalen, H. S. Forrest, and J. Myers* 701

BIOLOGY.—IONIC SEMIPERMEABILITY AS A BULK PROPERTY . . . . . *By Spencer L. Baird, Jr., George Karreman, Helmuth Mueller, and Albert Szent-Györgyi* 705

BOTANY.—THE EFFECT OF GIBBERELLIN UPON FLOWER FORMATION . . *By Anton Lang* 709

CHEMISTRY.—CHANGES IN PROTEIN TOPOGRAPHY UPON OXYGENATION . . . . . *By Irving M. Klotz and Richard E. Heiney* 717

CHEMISTRY.—VECTOR MODEL FOR INDIRECT PROTON HYPERFINE INTERACTIONS IN π-ELECTRON RADICALS . . . . . *By Harden M. McConnell* 721

CHEMISTRY.—CRITIQUE OF THE THEORY OF OPTICAL ACTIVITY OF HELICAL POLYMERS . . . . . *By William Moffitt, Donald D. Fitts, and John G. Kirkwood* 723

GENETICS.—A REMARKABLE ACTION OF THE MUTANT "RUDIMENTARY" IN *Drosophila Melanogaster* . . . . . *By Richard B. Goldschmidt* 731

GENETICS.—SPECIAL PROBLEMS INHERENT IN THE STUDY OF HUMAN GENETICS WITH PARTICULAR REFERENCE TO THE EVALUATION OF RADIATION RISKS . *By James V. Neel* 736

GENETICS.—THE SCOPE OF GENETICS . . . . . *By Curt Stern* 744

MATHEMATICS.—DYNAMIC PROGRAMMING AND STATISTICAL COMMUNICATION THEORY . . . . . *By Richard Bellman and Robert Kalaba* 749

MATHEMATICS.—SOLUTION OF THE BURNSIDE PROBLEM FOR EXPONENT 6. . . . . *By Marshall Hall, Jr.* 751

MATHEMATICS.—PARABOLIC EQUATIONS . . . . . *By John Nash* 754

PHYSICS.—RADIOACTIVE FALLOUT . . . . . *By Willard F. Libby* 758

ZOÖLOGY.—THE LOCALIZATION OF BASIC PROTEINS IN THE NUCLEI OF LARVAL *Drosophila* SALIVARY GLANDS . . . . . *By Edward C. Horn and Calvin L. Ward* 776

ZOÖLOGY.—A LABORATORY STUDY OF THE EFFECT OF REMOVAL OF NEWBORN ANIMALS FROM A POPULATION . . . . . *By L. Basil Slobodkin* 780